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A CYTOLOGICAL MONOGRAPH OF THE AMERICAN SPECIES OF TRADESCANTIA

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(WITH THIRTY-TWO FIGURES)

Introduction

In *Tradescantia*, as in all genera, there are certain special features of its evolutionary processes which are peculiar to itself and to its immediate relatives; certain general ones which are more fundamental. To sift the special from the general and to examine both, and to measure the evolutionary processes taking place in the American species at the present time, have been and still remain the objective of a comprehensive program of cooperative research. A taxonomic monograph of the American species has recently appeared (3), and a preliminary discussion (1) and isolated technical papers (2, 14, 19, 20, 21) dealing with certain phases of the problem have already been published. The following pages bring together a general cytological survey of the problem and a discussion of the bearing of this evidence.

Chromosome number and morphology in the Commelinaceae

An evaluation of the phylogenetic importance of the various evolutionary processes in *Tradescantia* will have greater significance if preceded by a cytological survey of the Commelinaceae as a whole. Such a survey should yield useful evidence as to the importance of structural changes in the chromosomes. The differentiation of species or even of genera may be dependent upon gene mutation with no

apparent change in chromosome structure or morphology; but in many cases differentiation is associated with structural and numerical changes in the chromosomes. These changes may include (1) autopolyploidy, the duplication of similar genoms; (2) aneuploidy, the duplication of only a few chromosomes of the genom; (3) segmental interchange between non-homologous chromosomes which may change the morphology of the genom; (4) fragmentation and loss of chromosome segments in polyploids; (5) inversion or translocation of segments; and (6) changes in chromosome size. Some of these numerical and structural changes may be directly effective in differentiation, but their chief function is providing the initial isolation permitting independent development of genic changes. Another important factor in differentiation is (7) allopolyploidy, the union and duplication of unlike genoms. This process involves a simultaneous change in genetic constitution and chromosome number.

The Commelinaceae are especially favorable material for a cytological study of the differentiation of genera and species. Many of the genera have been studied in some detail by DARLINGTON (**6**), who reviews the rather meager literature on the cytology of this group of plants. Unfortunately the family is much in need of comprehensive monographic treatment. The most recent attempt (**5**), while a great advance, is admittedly incomplete. A general discussion of evolutionary trends within the family, in the light of the cytological evidence, will scarcely be possible until the delimitation of the genera and their logical classification have been more adequately considered.

We have made a cytological study of nine genera and thirty-two species. To these can be added the information obtained by DARLINGTON, bringing the totals to thirteen genera and about thirty-seven species. The analysis of the genus *Tradescantia* has been done in considerable detail, and all the species native to the United States have been examined, with the exception of *T. wrightii* and *T. pinetorum*.

The data on chromosome number and morphology have been obtained from aceto-carmine smears of microsporocytes and developing microspores. The microspore division is especially favorable for study, because the metaphase plate is relatively flat and the single genom is present. The chiasma frequency at meiosis and pollen fer-

tility have been determined for several species, for artificial F_1 hybrids, and for plants from hybrid colonies.

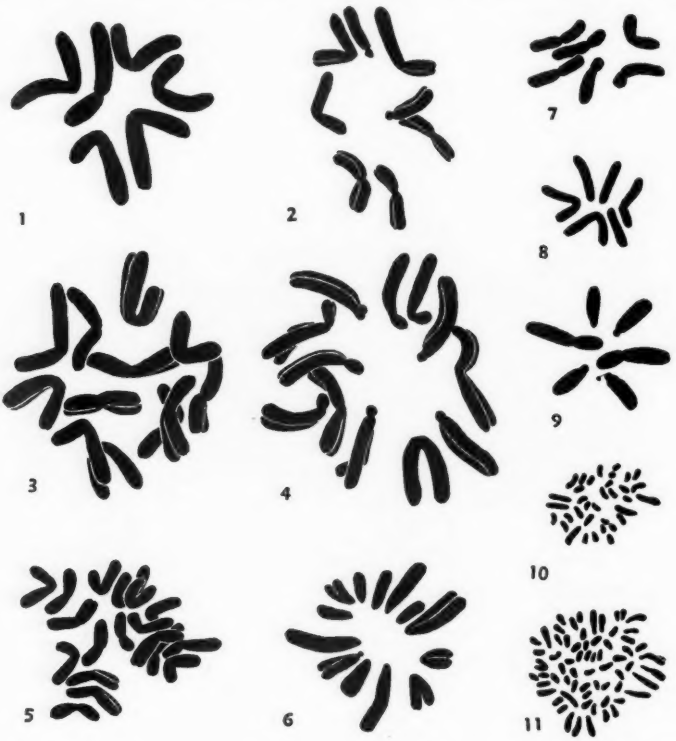
The common Tradescantias of the United States belong to a group of species all closely related to *T. virginiana*. The diploid forms have six pairs of large chromosomes, while the tetraploids have twelve pairs of chromosomes. The following species are known only as diploids: *T. gigantea*, *T. humilis*, *T. edwardsiana*, *T. paludosa*, *T. ernestiana*, and *T. hirsuticaulis*. *T. bracteata* is usually diploid, but a triploid form has been found. Both diploid and tetraploid forms have been found in *T. hirsutiflora*, *T. occidentalis*, and *T. canaliculata*. *T. subaspera* var. *typica*, *T. subaspera* var. *montana*, *T. roseolens*, *T. virginiana*, *T. longipes*, *T. ozarkana*, and *T. tharpii* have been found only as tetraploids. It is probable that certain species listed as only diploid or only tetraploid may be found to contain both types as more individuals are studied. The diploid and tetraploid forms within a single species are very similar in morphological characters, and cannot be distinguished without cytological analysis.

The chromosomes of the typical Tradescantias have median or submedian fiber constrictions (figs. 1, 3, 24). There is no consistent difference in the chromosome size of different diploid species, or within the tetraploid forms, but the tetraploids in general have somewhat shorter chromosomes than have the diploids.

Segmental interchange has been found in occasional plants of four diploid species, *T. edwardsiana*, *T. humilis*, *T. canaliculata*, and *T. gigantea*. Reciprocal translocations had occurred between two nonhomologous chromosomes. The interchanges were approximately equal so that the microspore chromosomes were indistinguishable from those of normal plants, but at meiosis the interchange plants form a ring or chain of four chromosomes and four bivalents (fig. 20). The segregation of the chromosomes in the interchange ring results in about 50 per cent pollen sterility (19). Segmental interchange was also found in certain tetraploid species, and rings of six or eight chromosomes were observed; but the cytological complexity of the tetraploid prevents ready analysis of interchanges by cytological observation through the failure of any consistent behavior of interchange chromosomes.

Chromosome fragments have been found in both diploids and

tetraploids. These are usually small chromosome segments with a terminal or subterminal fiber attachment point (fig. 21). They are rather regular in behavior in both mitotic and meiotic cells and are transmitted by both egg cell and pollen grains. The number of frag-

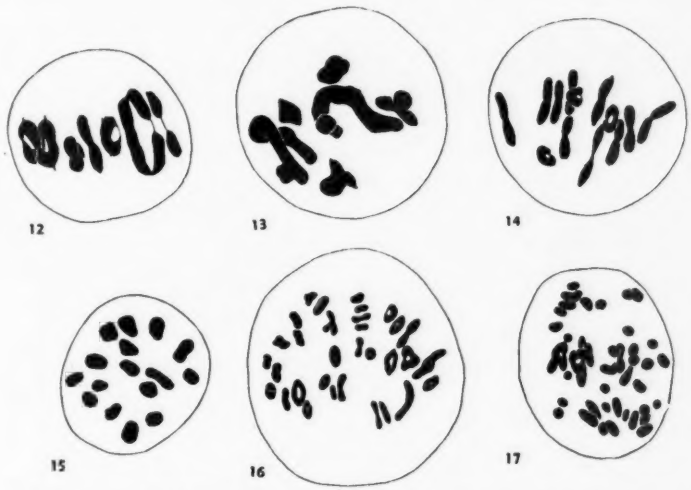


FIGS. 1–11.—Chromosomes of microspore nuclei in species and genera of Commelinaceae. From aceto-carmine smears: Fig. 1, *Tradescantia humilis;* fig. 2, *Tradescantia* sp. "Victoria"; fig. 3, *T. canaliculata;* fig. 4, *T. rosea;* fig. 5, *Setcreasea brevifolia;* fig. 6, *T. micrantha;* fig. 7, *Rhoeo discolor;* fig. 8, *Spironema fragrans;* fig. 9, *Callisia repens;* fig. 10, *T. geniculata;* fig. 11, *T.* sp. "bench." ×1200.

ments may vary from one to a dozen or more in diploid plants with little or no change in the appearance or the fertility of the plant. While particularly common in *T. paludosa*, they have been found in all the other species which have been extensively examined. A de-

tailed study of the behavior of the small fragments has been made by DR. T. W. WHITAKER (in press). Much larger fragment chromosomes have occasionally been found, particularly among plants from localities where specific hybridization was suspected.

The genoms of the tropical species of *Tradescantia* may differ from those of the Virginiana group in number, size, and morphology of the chromosomes. *Tradescantia* "Victoria" is an unidentified Mexican



FIGS. 12–17.—Meiotic divisions in genera of Commelinaceae: Fig. 12, *Tradescantia* sp. "Victoria," four bivalents and two quadrivalents; fig. 13, *Campelia zanonia*, four bivalents and two chains of four chromosomes each; fig. 14, *C. anomala*, thirteen bivalents; fig. 15, *Tradescantia geniculata*, sixteen bivalents; fig. 16, *Tinantia fugax*, about thirty-two bivalents; fig. 17, *Commelina benghalensis* with about six bivalents and thirty-six univalents. ×1000.

species allied to *T. geniculata*. The haploid chromosome number is eight, including six chromosomes with median fiber constrictions and two with subterminal constrictions (fig. 2). At meiosis there are usually two ring bivalents and two quadrivalent rings or chains of four chromosomes each (fig. 12).

There are several reasons for believing that this plant is an aneuploid with two duplicate chromosomes. In species with the basic number of chromosomes, such as the diploid Tradescantias, the

microspore nucleus does not divide unless it contains a complete genom. In Victoria the microspore nucleus may divide with only seven chromosomes, indicating that the absent chromosome has a duplicate homologue. If the quadrivalents were segmental interchange rings, random distribution would produce about 75 per cent sterility, but 94 per cent of the pollen is good. Therefore, the rings of four chromosomes must be quadrivalents, each with four homologous chromosomes. The frequency of bivalent formation also supports the assumption that these rings are quadrivalents. In normal tetraploids about one-third of the chromosomes pair as bivalents at meiosis, while in segmental interchange plants the interchange chromosomes seldom form bivalents. We may conclude then that Victoria is an aneuploid individual with four pairs of chromosomes and two quadrivalents.

Tradescantia rosea (*Cuthbertia rosea* Sm.) of southeastern United States has twelve pairs of large chromosomes, four of which resemble the chromosomes of species allied to *T. virginiana*, while eight have subterminal fiber constrictions (fig. 4). The reduction divisions are regular and the pollen fertility is normal.

Tradescantia micrantha of the Gulf Coast has thirteen pairs of chromosomes all of which have terminal or subterminal fiber attachment points. Four of the chromosomes are about as large as those of *T. rosea* but the others are much shorter (fig. 6).

There are sixteen pairs of chromosomes in *T. geniculata* and only bivalents are formed at meiosis (fig. 15). Four of the chromosomes have more or less median fiber constriction points while twelve are terminal or subterminal. According to DARLINGTON, *T. navicularis* also has sixteen pairs of chromosomes, but in this species all chromosomes have subterminal fiber constrictions. In both species the chromosomes are relatively large.

DARLINGTON found thirty pairs of small chromosomes in *T. fluminensis*. We have found about thirty-five pairs of small chromosomes in a species allied to *T. geniculata* (fig. 10), and about sixty-five pairs of chromosomes in an undetermined tropical species (fig. 11). In all these species with small chromosomes the fiber constrictions are usually terminal or subterminal.

The monotypic genus *Rhoeo* has twelve chromosomes in somatic

cells. At meiosis these twelve chromosomes are attached end to end to form a ring or one or more chains of chromosomes. In this segmental interchange ring no two chromosomes are completely homologous and the order of the chromosomes is always the same (fig. 23). The behavior of these chromosomes has been described in considerable detail (**15, 16**). Four of the twelve chromosomes have subterminal fiber constrictions while the other eight are more or less median (fig. 7). The high frequency of irregular distribution of chromosomes at meiosis results in about 80 per cent pollen sterility, but seeds are produced and the progeny breed true to type.

Spironema fragrans (fig. 8) and *Callisia repens* (fig. 9) have genoms with two median or submedian chromosomes and four with subterminal fiber attachments. Although the genoms are somewhat similar in chromosome morphology and chromosome size, the differences in plant size are about as extreme as can be found in the Commelinaceae. Aside from mere size, the genera are morphologically similar and are usually classified by taxonomists as being very closely related (**5**).

The chromosomes of *Setcreasea* resemble those of the Virginiana group of *Tradescantia* in respect to the location of the fiber constrictions, but they are somewhat smaller in size. DARLINGTON found twelve pairs of chromosomes in *Setcreasea brevifolia* (= *Treleasia brevifolia* Rose) while we find eighteen pairs of chromosomes in this species (fig. 5). At meiosis about half of the chromosomes are paired as hexavalents while the others usually form bivalents and quadrivalents. About 80 per cent of the pollen is good.

Zebrina pendula has twelve pairs of large chromosomes, ten of which have subterminal fiber attachments.

The monotypic genus *Campelia zanonia* has a basic number of eight and all the chromosomes are large. At meiosis there are four bivalents and two chains of four chromosomes each (fig. 13). These chains appear to be the result of segmental interchange rather than of chromosome duplication, although we have insufficient data to determine this point conclusively.

The haploid chromosome number is twenty-eight in *Commelina nudiflora* and about forty-five in *C. coelestis* (**6**). DARLINGTON found thirty-four chromosomes in *Commelina benghalensis*, but in the form

we have used there are about six bivalents and thirty-six univalents (fig. 17). In this plant there is complete pollen sterility. The fact that there are six bivalents indicates that there is no general suppression of meiosis and yet there are about thirty-six univalents. Such behavior would seem to indicate that the univalents include unlike genoms and that allopolyploidy is a factor in species differentiation in the Commelinaceae.

DARLINGTON has obtained chromosome counts of *Coleotrype natalensis* (n = 18), *Dischorisandra thyrsiflora* (n = 19), and *Tinantia fugax* (n = 32). Of these genera we have examined only *T. fugax* and find about thirty-two bivalents at meiosis (fig. 16). There are about forty bivalents in *Palisota bracteata*.

A list of the various genera and many of the species is given in table I. The haploid chromosome number (n) is given, together with the number of chromosomes with more or less median fiber constrictions and those with subterminal constrictions, and the approximate relative sizes—large (l), medium (m), or small (s). The counts were made by DARLINGTON (D) or by the junior writer (S). We have not included the individual species in the Virginiana group of Tradescantias because their genoms are morphologically similar.

GENERIC AND SPECIFIC DIFFERENTIATION IN THE COMMELINACEAE

All of the known factors which may be associated with taxonomic differentiation seem to be involved in the Commelinaceae. The differentiation of genera and species may be associated with gene mutation, autopolyploidy, allopolyploidy, aneuploidy, segmental interchange, or loss of chromosome segments.

The diploid species related to *T. virginiana* are apparently differentiated only by gene mutation. The genoms are similar in respect to chromosome number and chromosome morphology, and a cytological analysis of species hybrids shows that the genoms are similar in structure. Since these species are interfertile, isolation is a necessary factor in species differentiation. This isolation seems to have been effected by differences in ecological adaptation, as will be discussed later in this paper.

Autopolyploidy is also found in the Virginiana Tradescantias. Autopolyploidy in this genus has no direct relation to speciation

since tetraploidy induces little or no change in morphological characters in *Tradescantia*. Tetraploidy, however, may serve to isolate a new variant, since even intraspecific hybrids between tetraploids and diploids are about 50 per cent sterile. The tetraploids have a

TABLE I

CHROMOSOME NUMBER AND MORPHOLOGY IN THE COMMELINACEAE

GENUS AND SPECIES	CHROMOSOME NO.	FIBER CONSTRICTION		SIZE	AUTHORITY
		MEDIAN	SUBTERMINAL		
Tradescantia (Virginiana species)	6	6	.....	l	S
T. sp. "Victoria"	8	6	2	m	S
T. (Virginiana species)	12	12	.....	l	D, S
T. rosea Vent.	12	5	7	l	S
T. micrantha Torr.	13	.....	13	l	S
T. geniculata Jacq.	16	4	12	m	D, S
T. navicularis Ortgies	16	.....	16	l	D
T. fluminensis Vell.	30	.....	.....	s	D
T. "geniculata"	35	.....	.....	s	S
T. albiflora Kth.	36	.....	.....	s	S
T. sp. "bench"	65	.....	.....	s	S
Rhoeo discolor Hance	6	4	2	m	D, S
Spironema fragrans Lindl.	6	2	4	m	D, S
Callisia repens L.	6	2	4	m	S
Campelia zanonia H. B. K.	8	6	2	l	S
C. nudiflora L.	28	.....	.....	s	D
C. benghalensis L.	24–34	.....	.....	s	D, S
C. coelestis Willd.	45	.....	.....	s	D
Setcreasea brevifolia (Rose) K. Schum. et Syd.	12–18	12–18	.....	m	D, S
Zebrina pendula Schnitzel	12	2	10	l	D
Commelinantia anomala (Torr.) Tharp	13	3	10	m	S
Cyanotis somaliensis C. B. Clarke	14	3	11	Very s	D
Coeleotrype natalensis C. B. Clarke	18	4	14	s	D
Dischorisandra thyrsiflora Mikar	19	.....	.....	m	D
Tinantia fugax Scheidw.	32	.....	.....	s	D, S
Palisota bractiosa C. B. Clarke	40	.....	.....	m	S

longer blooming period than the diploids and have a higher survival value, as is shown by their greater distribution. The species known only as tetraploids may have been differentiated from their diploid ancestors by gene mutation, or the diploid ancestors may have been eliminated by natural selection, leaving only the tetraploid forms of these species.

The duplication of only a few of the chromosomes of the genom,

or aneuploidy, may be effective in speciation by producing an unbalanced relation between genes, or it may serve indirectly by effecting partial or complete isolation of the new variant. The species listed as Victoria is an aneuploid type with two duplicate chromosomes in the genom. A number of other aneuploids have been found in *Tradescantia* and other genera.

Changes in chromosome morphology can be effected in both diploid and polyploid species by segmental interchange or translocation of chromosome segments. Segmental interchange has been found in several diploid species of *Tradescantia*, but there is no evidence that it has been a factor in the differentiation of these species. In the polyploid species the differences in chromosome morphology may be attributed to segmental interchange or loss of chromosome segments. Seven of the twelve chromosomes of *T. rosea* have subterminal fibers and all of the thirteen chromosomes in *T. micrantha* have subterminal fiber attachments, while in the Virginiana tetraploids all chromosomes have approximately median fiber constrictions. In a polyploid, chromosome segments may be lost so long as the duplicate segment remains, so that many of the chromosomes might lose an entire arm as is possibly the case in *T. micrantha* and other species with heterobrachial chromosomes.

In polyploid species which have only bivalents formed at meiosis, as in *T. geniculata* and in *Commelinantia anomala*, allopolyploidy may be involved. Such an interpretation is also in accord with the behavior of univalents in *Commelina benghalensis*. Segmental interchange and loss of segments in a polyploid would tend to produce the same result, however, although it seems improbable that multivalent chromosome association would be completely suppressed as it is in many of these polyploid species.

There is clear evidence of structural differences of the chromosomes in the different genera of the Commelinaceae. The diploid Tradescantias have six chromosomes, all of which have approximately median fiber attachments; in *Rhoeo discolor* two of the six chromosomes are somewhat subterminal, while in *Spironema fragrans* and *Callisia repens* four of the six chromosomes have subterminal fiber attachments. These differences appear to have originated by segmental interchange as is clearly the case in *Rhoeo*.

In the Commelinaceae the chromosomes of different genera, or even of different species, may differ greatly in size. It seems improbable that the number of essential genes varies greatly in different diploid genera. The width of the chromosomes is rather closely associated with their length. The length of the meiotic spireme in *Rhoeo* is as great as it is in the diploid Tradescantias, but the meiotic metaphase and microspore metaphase chromosomes of *Tradescantia* are almost twice as long as those of *Rhoeo*. The differences in chromosome size may be caused by the amount of chromatin produced by the gene string during the chromosome cycle. A small amount of chromatin would permit a closer association of chromomeres and a more compact coiling of the gene string, resulting in metaphase chromosomes decreased in both length and width. Within a species the chromosomes have the same diameter regardless of their length, and even the fragment chromosomes in *Tradescantia* form a chromonema coil or partial coil which is as wide as the gyres of chromonemata in normal chromosomes.

Chromosome pairing in Tradescantia species

The pairing of homologous chromosomes at meiosis has been studied in considerable detail. This study was made in order to obtain additional information on the mechanism of meiosis and to aid in the analysis of species hybrids.

The prophase stages of meiosis are difficult to follow, but there seems to be complete pairing of the chromosomes at pachytene. We have not been able to make any accurate study of early chiasma formation. Occasionally diplotene or early diakinesis chromosomes seem to show a number of interstitial chiasmata, but these may be overlaps or temporary adhesions. At metaphase the chiasmata are largely terminal or subterminal.

The study of chromosome structure and behavior at metaphase has permitted an interpretation of the earlier stages which cannot be determined directly. The interpretation is based on the behavior of small fragments, location of chiasmata, and the coiling of the chromatids at metaphase and anaphase.

Whitaker (in press) has found that small fragments with terminal fiber attachments pair almost as regularly as the normal biva-

lents which are about seven times as long. There is good evidence that these fragment chromosomes are duplicates of median segments of the normal genom. If chiasma formation is at random one might expect a fragment to be paired near the spindle fiber region of a normal chromosome, as MATHER (12) has found in *Lilium*. But in the diploid plants of *T. paludosa* examined, fragments were never found paired with a major chromosome.

The chiasmata are almost always terminal in tetraploid Tradescantias and about 80 per cent are terminal in most diploid species. The nature of the coiled chromatids indicates that the prevalence of terminal chiasmata is not due to the terminalization of interstitial chiasmata. In plants with terminal chiasmata, such as *Tradescantia*, *Rhoeo*, *Secale*, and *Gasteria*, the direction of coiling of the chromatids at the first meiotic anaphase is seldom reversed (17), but in *Trillium* (8), *Lilium* (9), and *Vicia* (SAX unpublished), where interstitial chiasmata are frequent, the direction of coiling of chromatids is reversed rather frequently. Evidently the direction of coiling on either side of a chiasma is at random, so that the changes in direction of coiling of anaphase chromatids should be approximately twice the chiasma frequency at metaphase.

The failure of pairing between the small fragments and major chromosomes, the prevalence of terminal chiasmata, and the rare reversal of coiling in *Tradescantia* chromosomes can be explained on the assumption that the distal ends of chromosomes pair early enough to insure chiasma formation or at least to insure an intimate association of the distal ends of homologous chromosomes; but by the time the median regions are paired chiasma formation is inhibited. The pairing of normal chromosomes at metaphase is largely dependent on the terminal association of the bivalents. In the fragment chromosomes, complete association occurs early enough to insure regular pairing at meiosis, since the effective pairing length is almost as great as in the major chromosomes. This interpretation of chromosome pairing in *Tradescantia* explains the prevalence of proximal interlocking of non-homologous bivalents, and invalidates the use of the data as evidence in favor of the classic theory of chiasma formation, a possibility suggested by the writers (19) and reiterated by MATHER (12).

Chiasma frequency even within a single species of *Tradescantia* may be affected by environmental conditions or, presumably, by genetic factors. Preliminary experiments have shown that when plants are subjected to sudden temperature changes the chiasma frequency is greatly reduced. A diploid plant of *T. canaliculata* was placed in a temperature chamber at 5°–10° C. for several days and then transferred to a temperature of 30°–35° C. for three days. Partial or complete asynapsis was produced by this treatment (fig. 19). Plants transferred directly from the greenhouse to the higher temperature showed no change in chiasma frequencies. Apparently rather extreme temperature changes are necessary to induce asynapsis, and there is some evidence that relative, rather than absolute, temperatures are involved. Chromosome pairing in *Rhoeo* seems to be more susceptible to temperature changes than in *Tradescantia* (17). There is some indirect evidence that temperature changes in nature may affect chromosome pairing. In certain Tradescantias, and especially in structural or genetic hybrids, the pollen fertility may be relatively high during a period of good weather, but after a few cold wet days during the summer months, the pollen fertility may decrease considerably.

The genetic factors in chiasma frequency are indicated by the consistent variation within and between species, and the frequency in species hybrids. The data obtained from five diploid species are shown in table II, and include the number of microsporocytes examined, the average chiasma frequency per bivalent, and the percentage of interlocking between non-homologous chromosomes. The data on *T. paludosa* were obtained by E. D. KING. In the same species the average chiasma frequency may vary considerably in different plants. For example, in plant 12 the chiasma frequency is 2.5 while in plant 13–11 the average chiasma frequency is 1.7 per bivalent. Each average was based on 600 cells or 3600 bivalents.

There are some consistent differences in chiasma frequencies of different species, although the variation in different plants of a given species may be considerable. The chiasma frequency per bivalent is about 2.5 in *T. gigantea* but is only 1.8 in *T. humilis*. The differences in the number of interstitial chiasmata in different species are especially clear.

The percentage of interlocking between non-homologous chromosomes varies greatly even in the same species. Apparently such variation is caused by minor environmental factors which do not affect chiasma frequencies. This interlocking in *Tradescantia* and other genera may be an important, although indirect, factor in segmental interchange (**19**).

The pairing of chromosomes in the polyploid Tradescantias is of special interest because this genus is one of the few which has multi-

TABLE II

CHROMOSOME PAIRING IN DIPLOID SPECIES

SPECIES	PLANT NO.	NO. CELLS	AVERAGE XTA PER BIVALENT	PERCENTAGE INTERLOCKING
T. paludosa	10	40	2.5	3
	11	24	2.4	7
	12	600	2.5	1
	12–1	100	2.2	1
	13–1	40	1.9	23
	13–2	40	1.9	14
	13–3	40	1.9	4
	13–4	20	2.2	0
	13–11	600	1.7	2
	14	120	2.3	27
	30	30	1.6	1
T. humilis	.....	100	1.7	5
		20	1.8	.....
T. bracteata	.....	40	1.9	.....
T. gigantea	8	100	2.4	17
	9	40	2.5	15
	10	20	2.6	5
T. canaliculata	.....	20	2.6	3

valent chromosome pairing and maintains a high degree of fertility in nature. An autotriploid form of *T. bracteata* has been examined by KING, and we have studied chromosome pairing in several autotetraploids. The data on chromosome pairing in polyploids are shown in table III, and include the number of cells examined, the chiasma frequency per chromosome, pollen fertility, and the number of univalents, bivalents, trivalents, and quadrivalents. The chiasma frequency per chromosome is used instead of the frequency per bivalent since so many of the chromosomes form multivalent associations

at meiosis. If chromosomes are associated only in pairs at pachytene, the pairing length in a triploid is no greater than in a diploid, while in a tetraploid the pairing length may be twice that of the diploid (7).

In the diploid species of *Tradescantia* the chiasma frequency ranges from about 1.8 to 2.5 per bivalent, or about 0.9 to 1.3 per chromosome. In the diploid form of *T. bracteata* the chiasma frequency is about 1.0 per chromosome while in the triploid form it is 0.8. The total chiasma frequency in the triploids is greater than in the corresponding diploid, although the pairing length of the chromosomes is the same if association at pachytene is only in pairs. The

TABLE III

CHIASMA FREQUENCIES IN POLYPLOID TRADESCANTIAS

SPECIES	NO. CELLS	XTA PER CHROMOSOME	POLLEN FERTILITY	I	II	III	IV
Triploid							
T. bracteata	100	0.8	47	63	63	537	.....
Tetraploid							
T. virginiana	20	0.8	89	.....	93	.....	72
Ts×3	33	0.9	85	1	174	1	110
Hillsboro (T. canaliculata×T. virginiana)	17	0.8	84	4	70	4	63

greater chiasma frequency in the triploid cannot be attributed to the more rapid pairing, thereby increasing the effective pairing length, because almost all chiasmata in the triploid are terminal (99.7 per cent as compared with 84 per cent of terminal chiasmata in the diploid form). Evidence has been presented which indicates that there is little or no terminalization of chiasmata in *Tradescantia* before anaphase.

The chiasma frequency per chromosome in the tetraploids is lower than in the diploids although the pairing length per chromosome is the same, if association is only by pairs at pachytene. Almost all chiasmata are terminal in the tetraploids. The reduction in chiasma frequency in the tetraploid may be caused by competition in pairing which delays pairing and inhibits chiasma formation.

The various forms of trivalents in the triploid have been described

by KING. In the tetraploids more than half of the chromosomes are paired in quadrivalents. These are usually in the form of chains or rings (fig. 18). In most cases the chromosomes are oriented so that adjacent chromosomes in the quadrivalents pass to opposite poles, although exceptions are frequent. In *T. virginiana* we have observed various types of quadrivalents including figure 8 forms, and ring and rod figures of various forms. Interlocking between bivalents and quadrivalents is frequent (figs. 18–24).

Pollen fertility is relatively high in both diploid and tetraploid species of *Tradescantia*. In the triploids about half of the pollen is

TABLE IV

SPECIES OF TRADESCANTIA ALLIED TO T. VIRGINIANA; POLLEN STERILITY

DIPLOIDS			TETRAPLOIDS		
SPECIES	NO. PLANTS	FERTILE POLLEN (%)	SPECIES	NO. PLANTS	FERTILE POLLEN (%)
T. canaliculata	3	90	T. canaliculata	10	77
T. occidentalis	12	94	T. occidentalis	22	89
T. edwardsiana	2	98	T. virginiana	2	89
T. humilis	3	90	T. subaspera montana	12	71
T. gigantea	14	82	T. subaspera typica	2	75

sterile, caused by irregular distribution of chromosomes. In the diploid species the pollen fertility usually exceeds 90 per cent. In the tetraploid forms the pollen fertility is somewhat lower, ranging from 70 to about 90 per cent. The pollen fertility of a number of species is shown in table IV, and two of the species include both the diploid and the tetraploid forms. The slightly greater sterility of the tetraploids is attributed to the somewhat greater irregularity in chromosome distribution at meiosis.

Hybridization

ARTIFICIAL HYBRIDS

In genera whose chromosomes are large enough to permit detailed observation, a cytological study of first generation hybrids should yield data of some phylogenetic significance. The pairing of chromo-

FIGS. 18–24.—Photographs from aceto-carmine smear preparations: Fig. 18, first meiotic division in *Tradescantia virginiana* showing quadrivalents and bivalents. ×1200; fig. 19, partial suppression of meiosis in *T. canaliculata* by abnormal temperature. ×1200; fig. 20, first meiotic division in a plant of *T. gigantea* showing segmental interchange chain of four chromosomes, interlocked ring bivalents, free ring bivalent, and rod bivalent. ×1500; fig. 21, pairing of chromosome fragments at meiosis in *T. paludosa*. ×1200 (from a permanent smear preparation made by Dr. T. W. WHITAKER); fig. 22, first meiotic division in F_1 hybrid of *T. hirsutiflora*×*T. canaliculata* showing high frequency of interstitial chiasmata. ×1200; fig. 23, segmental interchange chain of twelve chromosomes at meiosis in *Rhoeo discolor*. ×2000; fig. 24, haploid set of chromosomes at metaphase in microspore division of *T. canaliculata*. ×1200.

somes by homologous portions rather than by a generalized attraction seems to be well established as a working hypothesis, although a few exceptional cases have been described. On this hypothesis a study of metaphase configurations and an analysis of chiasma frequencies should demonstrate whether or not gross physical rearrangements of the genoms have taken place in the development of the species in question.

Most of the diploid species of *Tradescantia* are of southern origin and are not winter hardy in the northern states. It is comparatively easy, however, to grow them in the greenhouse, where they usually come into flower in February. It is difficult to emasculate the flowers in the bud without injury, but since all the species related to *T. virginiana* are self-sterile (2), they can be emasculated after the flower opens and before much pollen is shed. The flowers are pollinated immediately after emasculation and the seed ripens in about a month. An entire head is devoted to a single cross and pollinations are made on the flowers as they develop for a period of several days or longer. When the capsules begin to ripen the entire head is inclosed in cheesecloth to catch the seeds when the capsules explode. About three seeds are set per capsule and these do not germinate readily.

The hybrids produced by artificial crossing of diploid species are listed in table V, with the number of microsporocytes examined, the average chiasma frequency per bivalent, and the percentage of pollen fertility. The chromosomes of the F_1 hybrids pair about as regularly as do those of the parental species. The average chiasma frequency of *T. paludosa* is about 2.1 per bivalent and is 1.8 for *T. humilis*. In the F_1 hybrids between these species the average chiasma frequency is 1.8 to 1.9. In the case of *T. canaliculata*×*T. paludosa* the chiasma frequency of the parents is about 2.6 and 2.1 respectively, while for the F_1 it is 2.0. In the cross *T. canaliculata*×*T. gigantea* the F_1 chiasma frequency is 2.2 as compared with 2.6 and 2.5 respectively for the parental species. Somewhat similar results were obtained from the cross between *T. paludosa* and a natural hybrid between *T. humilis* and *T. canaliculata*, known in our records as "Oakhill."

In these hybrids there is, in general, a somewhat lower chiasma frequency in the F_1 than is obtained by averaging the chiasma frequencies of the parental species, and in some cases the F_1 chiasma fre-

quency is lower than that of either parent. In view of the variation of individual plants within a species, the comparisons of chiasma frequencies of parental species and of the F_1 hybrids may not be significant, although the hybrids are consistently lower than the parental averages. In general, however, the behavior of the F_1 chromosomes at meiosis would never suggest their hybrid origin. Meiotic pairing is usually regular with only occasional univalents; there is no evidence of segmental interchange rings or chains; and the distribution

TABLE V

TRADESCANTIA HYBRIDS

Species	No. cells	Xta per bivalent	Pollen fertility (%)
Artificial hybrids			
T. paludosa×T. humilis	30	1.8	66
T. paludosa×T. humilis	100	1.9	.....
T. hirsutiflora×T. canaliculata	30	2.9	73
T. canaliculata×T. paludosa	30	1.9	47
T. canaliculata×T. paludosa	100	1.9	.....
T. canaliculata×T. paludosa	200	2.0	40
T. canaliculata×T. paludosa	100	2.2	.....
T. canaliculata×T. gigantea	100	2.2	40
T. paludosa×"Oakhill"	30	1.6	41
Natural hybrids			
T. canaliculata×T. humilis (Oakhill)	30	1.6	74
T. canaliculata×T. humilis (Oakhill)	240	1.9	.....
T. canaliculata×T. humilis (Oakhill)	40	1.7	.....

and behavior of chiasmata are essentially the same as in the parental species.

In one of the species hybrids, *T. hirsutiflora*×*T. canaliculata*, the F_1 chiasma frequency is relatively high and exceeds that of any of the diploid species which we have examined (fig. 21). Unfortunately we had only a single plant of *T. hirsutiflora* and did not obtain its chiasma frequency before it was discarded. The chiasma frequency of *T. canaliculata* is among the highest found—2.6 per bivalent; but for the F_1 the frequency is 2.9. It seems unlikely that the other parent would have a chiasma frequency greater than 2.9.

Although the chromosome behavior in F_1 plants shows little evidence of hybridity, the pollen sterility does show clearly that there are incompatible factors involved in these hybrids. The pollen fer-

tility of diploid species usually exceeds 90 per cent, but in the hybrids the pollen fertility ranges from 40 to 74 per cent. Even in crosses between species which are somewhat similar in morphological characters, such as *T. canaliculata* and *T. paludosa*, the pollen fertility is less than 50 per cent.

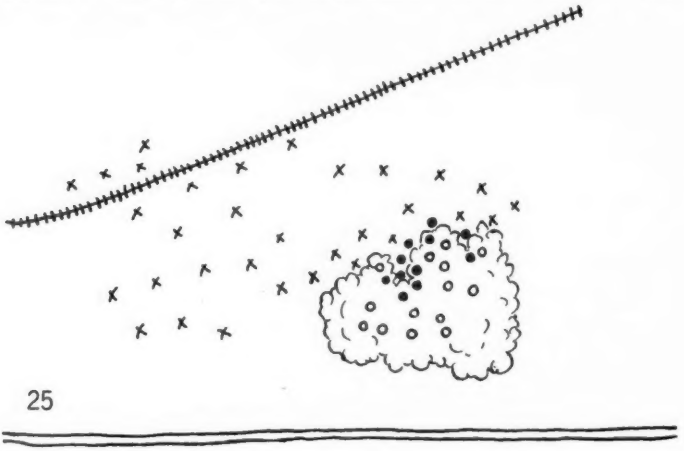
Since there is no evidence of structural changes in the chromosomes of different species, as indicated by the behavior of the chromosomes in F_1 hybrids, the differences between species must lie primarily in differences in genetic constitution. When structural changes occur in a species, as we have found in six plants, they are easily detected in the heterozygous condition by the formation of segmental interchange rings or chains of four chromosomes. It is possible that minute structural alterations would occur so that interchange rings would not be formed; but in all plants of pure species which have had a consistently low pollen fertility, we have found cytological evidence of structural changes in the chromosomes. The evidence seems to indicate that the low pollen fertility of the F_1 species hybrids is caused by the segregation of incompatible genetic combinations in the F_1 microspores.

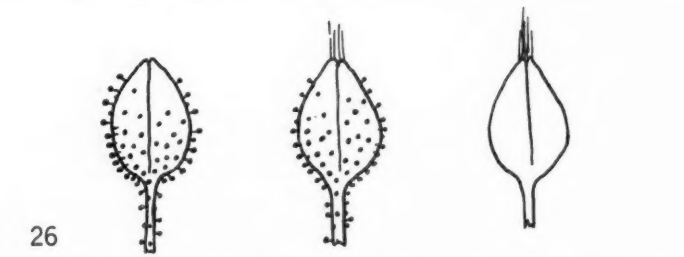
The combination of different genetic factors in the F_1 hybrids may also decrease the chiasma frequencies. Even when the genoms are similar in both morphological and genetical constitution, a genetic factor may inhibit chromosome pairing at meiosis as it does in certain cases in *Drosophila*, *Zea*, and *Triticum*. The degree of chromosome pairing may be greater in a generic cross than in a species cross, as PETO (13) has found in *Lolium* and *Festuca*. Such differences are evidently caused by combinations of genetic factors which influence meiosis, rather than by the degree of genetical and structural differentiation in the parental genoms. If these genetic factors cause asynapsis by delaying pairing long enough to inhibit chiasma formation, then one might expect that certain combinations of genetic factors would increase chiasma frequency by accelerating pairing. Such an effect in *Tradescantia* would increase the frequency of interstitial chiasmata. Possibly the high chiasma frequency found in the cross *T. hirsutiflora*×*T. canaliculata* is caused by genetic combinations which accelerate pairing of homologous chromosomes.

Natural hybrids

During the course of the investigation, numerous cases of natural hybridization were found between various species in the group related to *T. virginiana*. The interested reader is referred to the taxonomic monograph by Anderson and Woodson (3), where all the suspected cases are tabulated. Interspecific hybridization is apparently frequent in this group. During the last ten years the senior writer has been engaged in a study of the species problem, notably in the genera *Iris*, *Aquilegia*, *Tradescantia*, and *Uvularia*. In his experience interspecific hybridization is more general in *T. virginiana* and its near relatives than in any of the other genera studied intensively. It is planned, therefore, to make a detailed investigation of natural hybrids in *Tradescantia*, and the following is scarcely even a preliminary report on the subject. The senior writer will be glad to hear of any cases of suspected hybridization in *Tradescantia* or to assist in any way with the investigation of such cases. The results of interspecific hybridization, even between the same parental species, may be quite different, depending among other things upon the comparative frequency of the two species, the presence of polyploid races within either species (or both), and the length of time during which hybridization has been occurring. The following case of hybridization at Oakhill, Austin, Texas, is presented not so much because it is generally typical as because it has been studied more intensively than have any of the other cases which have been investigated to date. The hybrids at Oakhill occurred in the edge of a small copse of *Ilex*, scrub oaks, etc. (fig. 25). The more or less abandoned fields surrounding the wood were dry and stony, with numerous cacti. In the field along the neighboring railroad right of way, *T. humilis* was abundant. Well within the wood, *T. canaliculata* (=*T. reflexa*) (diploid race) was fairly common. Along the margin of the wood, particularly in one or two semi-shaded spots near the edge, were a number of intermediate plants. Some of them were clearly intermediate, others resembled one of the species much more than the other. Samples of all three types were collected and forwarded to the greenhouses of the Bussey Institution where they were subjected to cytological and morphological analysis over a period of two years (3).

The evidence may be summarized by saying that the plants thought to be *T. canaliculata* and *T. humilis* were cytologically regular and agreed morphologically with other representatives of these two species collected from nearby colonies where hybridization was not taking place. The suspected hybrids presented a number of cytological





FIGS. 25, 26.—Fig. 25, relationships of *T. humilis* (crosses), *T. canaliculata* (open circles), and intermediates (solid black) at Oakhill, Texas. Fig. 26, sepal pubescence (somewhat diagrammatic) in, left, *T. occidentalis;* right, *T. canaliculata;* center, *T. occidentalis* within range of *T. canaliculata.*

abnormalities often associated with hybridization, namely, high percentages of sterile pollen, micro-nuclei in the tetrads, etc. Certain of the hybrids were furthermore morphologically identical with the artificial hybrids raised between the same two species, and the whole case may be taken as proved.

Since the American species of *Tradescantia* maintain themselves as recognizable units, in spite of fairly frequent hybridization, it is obvious that there are barriers which prevent more frequent admixture. A few of these can be enumerated. In the first place, as might have been suggested by the cytological evidence alone, there seems to be a complete, or practically complete, barrier to crossing between the three groups of American species, that is between (1) *T. micrantha*, (2) *T. virginiana* and its relatives, and (3) *T. rosea*. Within the Virginiana group, chromosome number is apparently important. *T. bracteata*, a diploid, is associated mainly with tetraploid races or species and hybridization is rare. *T. hirsuticaulis*, a diploid, occurs rather commonly in association with the tetraploid *T. subaspera* var. *montana*, but in spite of extensive search Dr. HIRAM SHOWALTER found very few suspected hybrids (personal communication). Such hybrids would ordinarily be triploids and practically sterile. Another barrier between *T. hirsuticaulis* and *T. subaspera* var. *montana* is the time of blooming. The former species blooms very early, and, like most of the diploids, for only a short time. For this reason crossing with the later blooming species such as *T. virginiana*, *T. canaliculata*, and *T. subaspera* var. *montana*, with all of which it is occasionally associated, is kept at a minimum.

One of the most potent barriers is the difference in habitat preference. In the Oakhill example it was the distinctive preferences of *T. humilis* and *T. canaliculata* which confined successful hybridization to a narrow zone between the sunny field and the shady wood. Farther north in the middle west *T. canaliculata* grows characteristically in a hot, dry place, often on limestone outcrops. *T. subaspera* var. *typica* is general throughout the same region but for the most part is confined to acid or subacid soils in shade or semi-shade. Over a very wide area the two species are found within a comparatively short distance of each other but are seldom found growing together because of this pronounced difference in habitat preference. In the rare instances when they do occur near together hybrids can almost always be found.

The consequences of hybridization will vary greatly with the relative abundance of the two species, with the relative size of the zone of contact, and with the presence or absence of polyploid races within

either species or both. The last point has been discussed in detail in a recent publication (3) and need not be repeated here. If the zone of contact is relatively narrow and the two species are approximately equally abundant within this zone, we shall have the simplest possible relation between two hybridizing species; two well marked species connected by a comparatively few obvious, semi-sterile intermediates. If the zone is wider the percentage of intermediates may become so great that it is no longer possible to separate the two original entities, and they will probably most readily be classified as varieties of a single species. If one of the two species is much more abundant than the other in the zone of contact, the majority of the crosses which take place will be between previous crosses (or their descendants) and the commonest parent. Such individuals, three-fourths or more of their parentage having come from the predominant parent, will not ordinarily appear as hybrids but as somewhat extreme or unusual examples of the predominant species. An interesting example of this phenomenon is apparently to be found in the relation between *T. occidentalis*, the common spiderwort of the great plains, and *T. canaliculata* (*T. reflexa*), the common species of the middle west. Both species have glaucous foliage; *T. canaliculata* is glabrous except for a tuft of eglandular hairs at the tips of the sepals (fig. 26). Outside their zone of contact *T. occidentalis* has no terminal tuft, its pubescence is glandular (fig. 26) and tends to be strongest at the base of the sepal rather than at the tip. In their zone of contact, however, *T. occidentalis* is characteristically provided with at least a few terminal eglandular hairs, a feature obviously derived from *T. canaliculata*. We apparently have here an example of a phenomenon which, for the want of a better name, may be termed "mass infection." Hybridization has continued so long and so extensively that throughout the zone of contact the germplasm of the predominant species is "infected" with a small percentage of the germplasm of the less abundant species.

Cytological survey of American Tradescantias

Technique

To those whose acquaintance with *Tradescantia* is limited to the study of slides in the cytological laboratory, the assembling and

labeling of a collection of native Tradescantias might seem too simple a matter for scientific discussion. Experience has proved, however, that such is unfortunately not the case, and since the method finally adopted is comparatively simple and reasonably accurate, it may be briefly described. Tradescantias arrived at all times of the year and from all sorts of correspondents; they belonged to nearly thirty different species, several of these previously undescribed. For purposes of identification each collection was given a name, usually the name of the donor or of the immediate locality from which they came, and each individual plant was given a separate number. "Swarthmore 1" to "Swarthmore 13," for instance, represent thirteen plants of *T. virginiana* collected by Dr. Thomas Kerr along Crum Creek near Swarthmore College, Swarthmore, Pennsylvania. It is perhaps worth mentioning that upon arrival plants were carefully examined so that only a single individual was given a single number. With most of the northern species this was a comparatively simple matter, but with some of the southern species, notably *T. paludosa*, it was necessary to wash all the soil from the roots and make a careful examination before the plants were potted.

Presentation of data

The living collections, labeled and numbered as described, are summarized in table VI, where they are arranged alphabetically according to collection name. For each collection there is given the collector's name, the approximate location, the specific identification according to Anderson and Woodson's monograph, and the chromosome number. To simplify the table, for those plants collected by R. E. Woodson the collector is designated as "W." and for those collected by Edgar Anderson as "A." To these various collectors we are profoundly grateful, most particularly to Dr. Hiram Showalter and to D. George Diehl, who made substantial contributions of pertinent data as well as providing the actual plants. Herbarium specimens of the collections have been made wherever possible. The first set is to be deposited at the Herbarium of the Missouri Botanical Garden, and duplicate sets, so far as they are available, will be sent to other representative taxonomic institutions.

TABLE VI

NAME	NOS.	SPECIES	CHROMOSOME NO.	LOCALITY	COLLECTOR
Alabama	1–3	T. sp.	6	Warrior River, Alabama	Miss Barbara Andrew
Algonquin	1	T. canaliculata	12	Algonquin (St. Louis) Missouri	A.
Algo flex	1	T. canaliculata	12	" " "	W.
Algo hybrid	1–3	T. canaliculata×T. virginiana	12	" " "	W.
Algovirg	1	T. virginiana	12	" " "	W.
Amana	1	T. canaliculata	12	Amana, Iowa	D. George Diehl
Antonio	1–2	T. canaliculata	12	Antonio, Missouri	A.
Air Mail	1	T. sp. (?)	6	Texas	B. C. Tharp
Ashland	1–6	T. subaspera typica	12	Ashland, Missouri	A.
Anniston	1	T. subaspera montana	12	Anniston, Alabama	Hiram Showalter
August	1	T. hirsutiflora	12	.....	W.
Austin	1–13, 7a	T. occidentalis	6	Austin, Texas	A.
Balsam Gap	1–16	T. subaspera montana	12	Balsam Gap, North Carolina	A.
Beebe	1–9	T. occidentalis×T. canaliculata	12	Beebe, Arkansas	W.
Belew	1–3	T. occidentalis	6	Harmon, Oklahoma	Mrs. N. C. Belew
Bolton	1–4	T. canaliculata	12	Bolton, North Carolina	A.
Borglum	1–2	T. canaliculata	12	Stone Mt., Georgia	Hiram Showalter
Buda	1–0	T. gigantea	6	Buda, Texas	J. A. Devney
Bull Creek	1–3	T. edwardsiana	6	Bull Creek, Texas	A.
Cliff Springs	1–18	T. gigantea	6	Near Austin, Texas	J. A. Devney
Columbia	1–8	T. roseolens	12	Near Columbia, So. Carolina	A.
Clarke	1	T. bracteata	6	Grinnell, Iowa	Miss Cornelia Clarke
Caddo	1	T. sp.	6	Caddo Parish, Louisiana	C. Dorman
Deam	.....	T. virginiana	12	Indiana	C. C. Deam
De Soto	1–6	T. virginiana	12	De Soto, Missouri	W.
Dexter	1	T. canaliculata	6	Dexter, Michigan	U. of Michigan Botanical Garden

TABLE VI—*Continued*

Name	Nos.	Species	Chromosome no.	Locality	Collector
Diamond Lake	1–2	T. canaliculata	6	Diamond Lake, Noble, Co., Ind.	C. C. Deam
Edwardsiana	1–20, 15a, 10a	T. edwardsiana	6	Edwards Plateau, near Austin, Texas	A.
Elk River	1–6	T. ernestiana	6	Noel, Missouri	W.
Ellsmere	1	T. occidentalis	12	Ellsmere, Colorado	H. Stoddard
Florissant	1–2	T. canaliculata	12	Florissant, Missouri	A.
French Broad Creek	1–6	T. canaliculata×T. subaspera montana	12	Near Knoxville, Tennessee	A.
Fort Valley	1	T. virginiana	12	Fort Valley, Virginia	Hiram Showalter
Gates	1–2	T. bracteata	6	Manhattan, Kansas	David Gates
Gentilly	1–13	T. paludosa	6	Gentilly, Louisiana	A. & W.
Gravel Pit	1–4	T. subaspera montana	12	Jacksonville, Alabama	Hiram Showalter
Grinnell	20A & 20B	T. bracteata	9	Grinnell, Iowa	D. George Diehl
Hamburg	61–64, 80–84	T. canaliculata	12	Hamburg, Missouri	A.
Hair	1–5	T. subaspera montana	12	Near Jacksonville, Alabama	Hiram Showalter
Hazelton	1	T. subaspera typica	12	Hazelton, Indiana	U. of Michigan Botanical Garden
Hermann	I, P	T. subaspera typica	12	Hermann, Missouri	A.
Hillsboro	1–8	T. virginiana×T. canaliculata	12	Hillsboro, Missouri	A.
Hoffmann	1	T. virginiana	12	Sigourney, Iowa	Gerald Hoffmann
Ilex Grove	20–31	T. occidentalis	12	College Station, Texas	A.
Jacksonville	1–4	T. hirsuticaulis	6	Jacksonville, Alabama	Hiram Showalter
Jasper	1–4	T. ernestiana	6	Jasper Co., Missouri	W.
Jeanette	1–6	T. occidentalis	12	College Station, Texas	A.
Jolivette	1–5	T. canaliculata	12	La Crosse, Wisconsin	Miss Eva L. Jolivette
Kellogg	23	T. bracteata	6	Kellogg, Iowa	D. George Diehl
Killarney	1–5	T. longipes	12	Iron Co., Missouri	W.

TABLE VI—*Continued*

NAME	NOS.	SPECIES	CHROMOSOME NO.	LOCALITY	COLLECTOR
Knoxville	1–6	T. subaspera montana	12	Knoxville, Tennessee	H. M. Jennison
La Barque Creek	1	T. canaliculata	12	La Barque Creek, Missouri	A.
Lake Cliff	.....	T. gigantea	6	Lake Austin, Texas	J. A. Devney
Louisville	1–5	T. subaspera typica	12	Louisville, Kentucky	D. George Diehl
Madison	1	T. canaliculata	12	Arena, Wisconsin	N. C. Fassett
Mangelsdorf	1–21	T. occidentalis	12	College Station, Texas	P. C. Mangelsdorf
Manhattan	1	T. bracteata	6	Manhattan, Kansas	U. of Michigan Botanical Garden
Marthasville	21–23	T. subaspera typica	12	Marthasville, Missouri	A.
Manitou	1	T. occidentalis	12	Manitou, Colorado	Herbert Stoddard
Mattese	1	T. virginiana	12	Mattese, Missouri	W.
Montgomery	1–6	T. hirsuticaulis	6	Montgomery Co., Arkansas	Delzie Demaree
Mountain Lake	1	T. canaliculata	12	Mountain Lake, Giles Co., Virginia	Hiram Showalter
Mt. Bonnell	1–10	T. gigantea	6	Mt. Bonnell, Texas	J. A. Devney
Mt. Mitchell	1–8	T. subaspera montana	12	Mt. Mitchell, North Carolina	A.
Moore's Station	6	T. sp.	6	Moore's Station, Iowa	D. George Diehl
McKee	1–11	T. canaliculata	12	State College, Mississippi	J. C. McKee
Mo. Pac	1–15, 4W, 4V, 4T	T. gigantea	6	Austin, Texas	A.
New Harmony	1	T. subaspera typica	12	New Harmony, Indiana	U. of Michigan Botanical Garden
N.D. & D.	1–25	T. humilis	6	Austin, Texas	A.
North Slope	1–6	T. hirsuticaulis	6	Stone Mt., Georgia	A.
Oakhill	1–29	T. canaliculata×T. humilis	6	Oakhill Road, Austin, Texas	A.
Onion Creek	1–7	T. gigantea	6	Onion Creek, Austin, Texas	J. A. Devney
Orlando	1	T. canaliculata	12	Orlando, Florida	Woolman
Palmer	1	T. occidentalis	12	North Dakota	Palmer

TABLE VI—*Continued*

Name	Nos.	Species	Chromosome no.	Locality	Collector
Pana	1	T. canaliculata	12	Pana, Illinois	A.
Perry	1–2	T. hirsutiflora	12	Athens, Georgia	Miss Lily Perry
Pierson	1	T. bracteata	6	Pierson, Iowa	D. George Diehl
Portage des Sioux	31–47	T. bracteata	6	Portage des Sioux, Missouri	A.
Portland	1	T. virginiana	.....	Portland, Indiana	C. C. Deam
Quad petal	1	T. hirsuticaulis	6	White's Gap, Alabama	Hiram Showalter
Red Indiana	.....	T. virginiana	.....	Indiana	C. C. Deam
Roaring River	1	T. ozarkana	12	Roaring River State Park, Missouri	W.
Red Rock	1	T. canaliculata	12	Red Rock, Hughes Mt., Missouri	J. A. Steyermark
Reflexa	8	T. canaliculata	6	Austin, Texas	B. C. Tharp
River Cliff	1–10	T. gigantea	6	Austin, Texas	J. A. Devney
River Terrace	1–8	T. gigantea	6	Austin, Texas	J. A. Devney
Rocky Prairie	1–2	T. tharpii	12	Webb City, Missouri	W.
Royal	1	T. bracteata	6	Royal, Nebraska	U. of Michigan Botanical Garden
Royal Gorge	1	T. canaliculata	12	Royal Gorge, Missouri	J. A. Steyermark
Reita	1	T. canaliculata	12	Schoolcraft, Michigan	A. C. Anderson
San Antonio	1–5	T. sp. (?)	6	San Antonio, Texas	Mrs. S. D. McKelvey
Sand Mountain	1	T. canaliculata×T. hirsuticaulis	12	Muscle Shoals, Alabama	Hiram Showalter
Schoolcraft	1–3	T. canaliculata	12	Portage, Michigan	A.
Shoal Creek	1–14	T. gigantea	6	Austin, Texas	J. A. Devney
Signal	1–2	T. occidentalis	12	College Station, Texas	A.
Smith's Mills	1	T. subaspera typica	12	Smith's Mills, Kentucky	U. of Michigan Botanical Garden
Somena	1	T. occidentalis	12	Somena, Kansas	Herbert Stoddard
Starved Rock	1–3	T. canaliculata	12	Starved Rock, Illinois	Edward King
Steere	1–6	T. canaliculata	6	Ann Arbor, Michigan	W. C. Steere

TABLE VI—*Continued*

NAME	NOS.	SPECIES	CHROMOSOME NO.	LOCALITY	COLLECTOR
South Webster	1	T. canaliculata	12	Webster Groves, Missouri	A.
Swarthmore	1–13	T. virginiana	12	Swarthmore, Pennsylvania	Thomas Kerr
Showal	1	T. hirsuticaulis	6	Jacksonville, Alabama	Hiram Showalter
Sylva	1	T. subaspera montana	12	Sylva, North Carolina	Hiram Showalter
Tama	11–13	T. bracteata	6	Tama, Iowa	D. George Diehl
Tarbox	1	T. rosea	12	Brookgreen, So. Carolina	F. G. Tarbox, Jr.
Terrace	1–12	T. gigantea	6	Austin, Texas	J. A. Devney
Three Rivers	30	T. canaliculata	12	Three Rivers, Michigan	A.
Turkey Run	1–5	T. subaspera typica	12	Turkey Run, Indiana	Russell Artist
Texana	5, 5L, 5M	T. sp.	6	Austin, Texas	B. C. Tharp
Tocacco Landing	1	T. virginiana	12	Harrison Co., Indiana	C. C. Deam
Ullin	20	T. canaliculata	12	Ullin, Illinois	A.
Waterlco	1	T. canaliculata	6	Waterloo, Iowa	D. George Diehl
Warrenton	1–5	T. canaliculata	12	Warrenton, Georgia	A.
Washington University	31–34	T. canaliculata	12	St. Louis, Missouri	D. George Diehl
White's Gap	1	T. hirsuticaulis	6	White's Gap, Alabama	Hiram Showalter
Winter Haven	1–56	T. sp. (?)	6	Winter Haven, Texas	E. Mortensen
Winslow	1	T. occidentalis	12	Winslow, Arizona	Mrs. S. D. McKelvey

Summary of data on chromosome numbers

Broadly speaking, the American species of *Tradescantia* allied to *T. virginiana* can be divided into (1) diploid races and species, mostly southern and mostly of comparatively narrow range (fig. 27); and (2) autotetraploid species and races, mostly northern, several of them being of very wide distribution (fig. 28).

The following species are so far known only as diploids:

T. edwardsiana Tharp.—A species of very restricted distribution found in the Edwards Plateau in Texas (**23**). Morphological and geological evidence indicates it as probably one of the "oldest" elements among the American Tradescantias.

T. ernestiana Anderson and Woodson.—A species closely allied to the tetraploid *T. virginiana*, but restricted to a small area in the western Ozarks.

T. gigantea Rose.—A distinct and well differentiated species, restricted to a small area in central Texas but a common plant within that area. It is, incidentally, one of the best diploid Tradescantias for cytological and genetical experimentation.

T. paludosa Anderson and Woodson.—A species which roots at the nodes like many of the tropical Tradescantias. Restricted to the lower Mississippi delta. It is characterized by a high frequency of small fragment chromosomes in addition to the regular diploid complement.

T. bracteata Small.—This species has the widest distribution of any of the diploid species. It is possible that it may be a tetraploid on the northwestern border of its range since no material has been cytologically examined from that region. Like all the diploids it has a short blooming period and, since it afterwards dies down completely, it is well adapted to the hot dry summers of the western prairies.

T. humilis Rose.—A common species of eastern Texas where it is something of a weed. It apparently hybridizes more frequently with other species than do any of the other diploid species.

T. subacaulis Bush.—Restricted to a small area in east Texas.

T. hirsuticaulis Small.—A delicate montane species restricted to a narrow belt along the southern border of the Appalachians and to an isolated area on the western side of the Mississippi embayment in the

FIG. 27

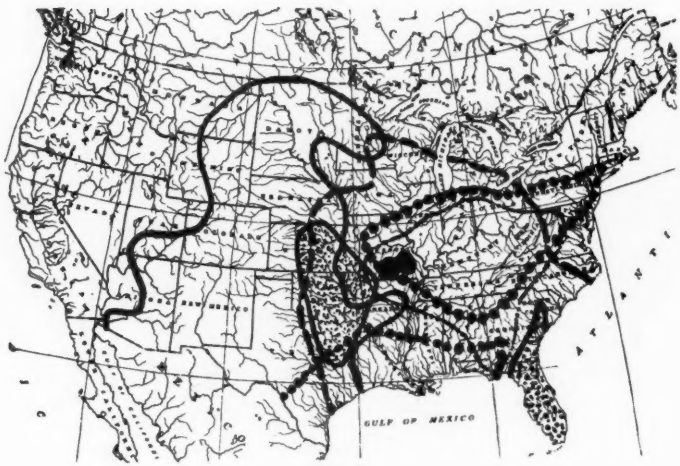


FIG. 28

FIGS. 27, 28.—Fig. 27 (above), approximate distribution of diploid species of *Tradescantia:* solid line, *T. bracteata* (middle west), *T. humilis* (Texas), *T. gigantea* (central Texas), *T. hirsuticaulis* (southern states). Stippled area, *T. paludosa* (Mississippi delta), *T. subacaulis* (Texas). Solid black area, *T. ernestiana* (Ozarks), *T. edwardsiana* (Texas). Fig. 28 (below), approximate ranges of tetraploid species of *Tradescantia:* Solid line, *T. occidentalis;* coarse dashes, *T. canaliculata;* large dots, *T. virginiana* in north and *T. hirsutiflora* on gulf coast; dash and dot, *T. subaspera;* stippled area, *T. tharpii* in middle west and *T. roseolens* in Florida; solid black area, *T. longipes.*

southern Ozarks. This is a range similar to many of the typical species of this region and indicates that they have been in the region since the close of the Cretaceous (**22**).

The following species are known only as tetraploids, or are predominantly so:

T. subaspera Ker-Gawl.—A widespread woodland and mountain species in the Appalachians and eastern Ozarks. Its probable relations with *T. canaliculata* are discussed in detail in the section on hybridization. Known only as tetraploid (fig. 29).

T. ozarkana Anderson and Woodson.—Restricted to a small area in the western Ozarks. Known only as a tetraploid.

T. virginiana L.—Few of the plants in cultivation under this name represent the genuine species (see below). It is a widespread woodland species in the central states. Known only as a tetraploid (fig. 30).

T. hirsutiflora Bush.—Found along the coastal plain of the Gulf of Mexico from Texas to Florida. It is a ubiquitous species, apparently hybridizing freely with other species within that area. A diploid in Texas and a tetraploid in the gulf states.

T. tharpii Anderson and Woodson.—A species of the southern great plains from Texas to Kansas. Known only as a tetraploid, although probably diploid in Texas.

T. canaliculata Raf.—A ubiquitous species, a common roadside weed in the middle west, actively spreading along railroad rights of way, etc. along the boundaries of its present range. A diploid in southern Texas, tetraploid in the north, although diploid individuals have been found at other points (fig. 31).

T. longipes Anderson and Woodson.—An "ancient" species with a very restricted distribution in the Ozarks. Closely allied to the more widespread and vigorous *T. tharpii*. Apparently always a tetraploid.

T. occidentalis (Britton) Smyth.—A widespread species of the great plains and eastern Rocky Mountains. A diploid in southern Texas, tetraploid elsewhere (fig. 32).

T. roseolens Small.—Closely allied to *T. occidentalis*, but native to Florida and the adjacent coastal plains. Known only as a tetraploid.

The approximate range of each species has been determined from the accurate distribution maps published in ANDERSON and WOOD-

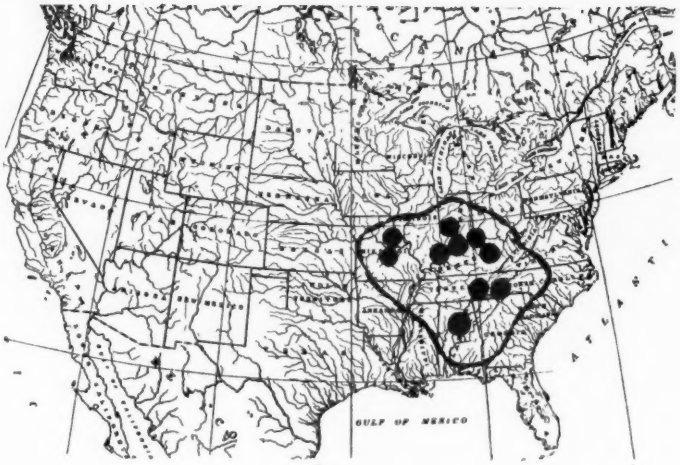


FIG. 29

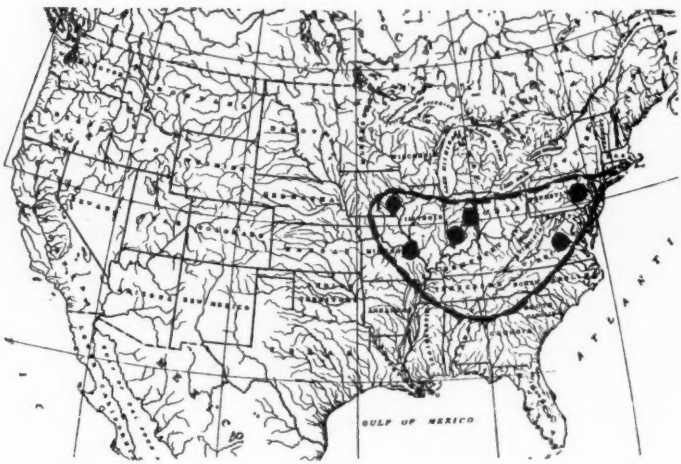


FIG. 30

FIGS. 29, 30.—Fig. 29 (above), approximate range (dotted line) of *T. subaspera* and localities from which plants were obtained for cytological examination. Fig. 30 (below), approximate range of *T. virginiana* and location of wild growing plants obtained for cytological examination.

FIG. 31

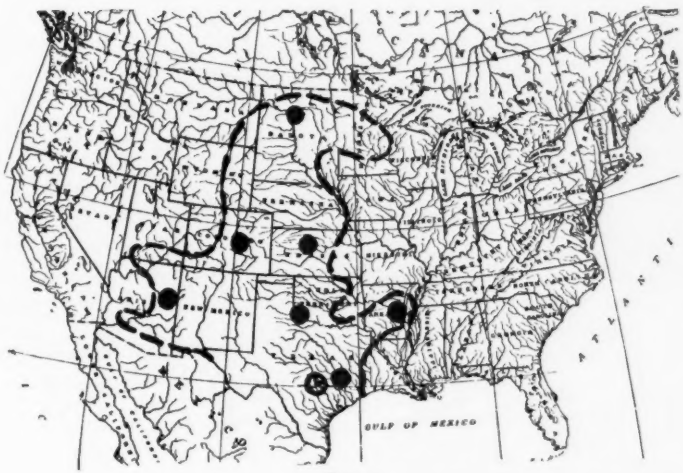


FIG. 32

FIGS. 31, 32.—Fig. 31 (above), approximate range (dotted line) of *T. canaliculata* and localities from which plants were obtained for cytological examination. Solid black, tetraploid; open circles, diploid. Fig. 32 (below), approximate range of *T. occidentalis* and localities from which plants were obtained for cytological examination. Solid black, tetraploid; open circles, diploid.

SON'S monograph (3) and is summarized in table VII. The average range in square miles for the diploid species is 83,475; for the tetraploid species, 376,300. Since the spreading power of a species is more accurately reflected by the radius of its distribution area rather than by the area itself, we have extracted the square roots of these two figures, obtaining an average "diameter" for the tetraploid species of

TABLE VII

TRADESCANTIA SPECIES ALLIED TO T. VIRGINIANA, WITH THEIR APPROXIMATE RANGES IN SQUARE MILES

	RANGE IN SQ. MILES
Species known only as diploids:	
T. edwardsiana	5,300
T. ernestiana	26,500
T. gigantea	10,600
T. paludosa	47,700
T. bracteata	381,600
T. hirsuticaulis	37,100
T. humilis	143,100
T. subacaulis	159,000
Species known only as autotetraploids, or predominantly so:	
T. subaspera	371,000
T. ozarkana	10,600
T. virginiana	397,500
T. hirsutiflora	265,000
T. tharpii	132,500
T. canaliculata	1,166,000
T. longipes	10,600
T. roseolens	79,500
T. occidentalis	1,166,000
Average range of diploids	83,475 sq. miles
Average range of tetraploids	376,300 sq. miles

613 miles and for the diploids of 289 miles. The ratio between these two diameters is 2.1 to 1; that is, a tetraploid species on the average has just about twice the radius of a diploid species.

Reports of intraspecific autopolyploidy in *wild* species, while not unknown (**4, 11**), are somewhat rare and for the most part are from genera, such as *Nasturtium*, in which vegetative propagation is well developed. A whole group of vigorous autopolyploid species, such as these Tradescantias, actively reproduced by seed (**1**) is therefore an

exceptional case. This section of the genus *Tradescantia* should therefore possess certain basic characteristics which make possible such an extensive and successful development of autopolyploidy. Two such characteristics may be suggested, terminal chiasmata and median attachment constrictions.

With completely terminalized chiasmata the configurations are fairly complicated, and occasionally lead to numerical non-disjunction, chromosome interchange, and other irregularities, but they are simple in comparison with what would have resulted with a high chiasma frequency and little or no terminalization. Under these latter conditions the configurations are obviously too complicated to permit regular disjunction. In this connection the range of *T. gigantea* is of interest. It is confined to an exceedingly small area in central Texas, yet within that area it exhibits many of the characteristics of a potential weed. It is, however, uniformly diploid. Extensive samplings of wild populations (93 plants from nine localities) have revealed only diploid plants. This is noteworthy in view of the fact that *T. gigantea* has a consistently higher chiasma frequency than *T. canaliculata* and *T. occidentalis* (table II). It seems not improbable that the absence of polyploid strains within *T. gigantea* may be due, not to their never having arisen, but that once originated their reduction divisions were too complicated to permit natural survival. A second characteristic of these Tradescantias permitting regular disjunction in autotetraploids is median or submedian attachment constrictions.

Consequences of autopolyploidy

It might confidently be predicted that autopolyploidy would produce individuals differing somewhat from the diploids from which they arose. Since from the simplest *a priori* assumptions some of the reactions of the nucleus are functions of its volume and some are functions of its surface, doubling the chromosome number (and hence doubling the volume, with a consequent increase in the surface of barely 60 per cent) should alter the general metabolic rate of the cell. As a matter of fact most autotetraploids differ from the diploids from which they arose, some of them markedly. In *Tradescantia* the difference is slight but perceptible. Two species, *T. canaliculata* and

T. occidentalis, have extensive diploid and tetraploid races which have been investigated during the course of this investigation. We have never been able to classify plants of either species as diploids or tetraploids by their appearance alone. Once classified by cytological examination, however, it has always been easy to see that the tetraploids are on the whole definitely larger and that they have much longer blooming seasons. This has been equally true of plants cultivated in the breeding plots during the summer and of those grown in pots in the greenhouse during the winter. As a consequence of their greater vigor the tetraploid species and races have a much greater colonizing ability, as has already been demonstrated. The cases of *T. canaliculata* and *T. occidentalis* are particularly interesting, each apparently having originated in the south as a diploid while autopolyploid races subsequently arose in each species and spread extensively over more than a million square miles, *T. occidentalis* on the great plains and *T. canaliculata* on the prairies and along the coastal plain. Each is a common weed in its own territory and is actively enlarging its boundaries at the present time. Most of the other tetraploid species present a similar picture, notably *T. hirsutiflora* which, a diploid in central Texas, has spread north and east as a tetraploid weed.

This entire section of the genus *Tradescantia* presents therefore an interesting example of parallel evolution. Endowed through their common origin with the same basic abilities, many of the species have evolved in a similar fashion. With genoms characterized, among other things, by median attachment constrictions and terminal chiasmata, several of the species have been able, independently, to produce vigorous autotetraploid races which were prepared to travel even faster and farther when white civilization appeared in North America.

Comparison of cultivated and indigenous Tradescantias

With many cytologists and geneticists the opinion has been prevalent that there is no real distinction between cultivated plants and wild species, or at least between cultivated plants run wild and the indigenous elements of a flora. While the question has seldom been argued in print, it has (outside the Scandinavian countries, at least)

been tacitly taken for granted as a working hypothesis by many if not most cytogeneticists. Certainly the senior writer has argued the question frequently and fruitlessly with many of his cytogenetic colleagues. It is gratifying, therefore, to be able to present not only the taxonomic evidence and the *a priori* reasoning which led him originally to his own point of view, but also a body of cytological facts, statistically summarized and pointing to the same conclusion.

The taxonomic evidence may briefly be summarized by saying that the majority of taxonomists consider cultivated plants as belonging to a somewhat different category from the strictly indigenous elements of a flora. Reasoning *a priori* from the known facts of cytology and genetics one might reach similar conclusions. Certainly the percentage of recessives, the opportunities for interspecific hybridization, for chromosome interchange, and for the survival of most cytological irregularities would be greatly different among cultivated plants from what obtained in the areas from which these plants were ultimately derived.

Fortunately in *Tradescantia* we have a remarkably complete test case. The common garden spiderwort is known to have been derived *in toto* from the American species closely related to *T. virginiana*, that species and *T. subaspera* Ker-Gawl. (= *T. pilosa* Lehm.) having been the most important contributing species. In the more than 300 years since their introduction to cultivation they have had the opportunity in European gardens, away from their original home, to run the usual course of a cultivated ornamental; to suffer periods of fashion and periods of neglect; to be consciously and unconsciously hybridized and selected, and to run wild again at certain points. In 1930 a representative sample of these cultivated spiderworts was brought together and analyzed cytologically (6). With the resources of the John Innes Horticultural Institution, the collection was a representative one and the cytological work is likewise unimpeachable. It forms an unusually trustworthy body of evidence, therefore, with which to compare the data on wild spiderworts compiled in the course of this investigation. The comparison (table VIII) is made by percentage frequencies for the various cytological types in the cultivated English plants on the one hand (*loc. cit.* p. 218) and for some 268 wild American plants for which definite records had been kept. Table

VIII shows how the cultivated plants give an erroneous impression of the percentages of triploids and of extra-chromosomal types, and fail to reveal the important diploid species. The interested reader is referred to the original paper (**6**) from which the data on the cultivated European plants were taken. It will be seen that although the investigation was avowedly (*loc. cit.* p. 207) an attempt to apply cytological methods to taxonomic questions, the failure to discriminate between the cultivated "*T. virginiana* L. U.S.A." and its wild progenitors led to erroneous conclusions as to the importance of vege-

TABLE VIII

COMPOSITION OF WILD AND CULTIVATED TRADESCANTIAS. PERCENTAGE COMPOSITION OF T. VIRGINIANA AND ITS CLOSE RELATIVES AS INDICATED BY WILD AND BY CULTIVATED PLANTS

	PERCENTAGE	
	CULTIVATED	WILD
2n	0.0	55.6
3n	5.3	0.4
4n	57.9	41.0
4n+f	26.3	3.0
4n+1	5.3	0.0
4n+1+f	5.3	0.0

tative reproduction (p. 254) and the relationships of the Texas species (p. 279).

The conclusion seems inescapable that the cultivated spiderwort, the so-called *T. virginiana* of European gardens, is quite a different assemblage cytogenetically from the native American species of *Tradescantia* from which it was ultimately derived. If this is true of *Tradescantia*, cultivated only 300 years, how much more different cytogenetically from their ultimate wild progenitors must be our common domesticated plants and animals.

Summary

1. Autopolyploidy, aneuploidy, segmental interchange, fragmentation, and differences in chromosome size were found in the nine

genera and thirty-two species of the Commelinaceae which were investigated cytologically.

2. Chromosome pairing has been studied in five diploid species of *Tradescantia*. The average number of chiasmata per bivalent is found to be fairly constant for any one plant but varies widely from plant to plant, even within the same species.

3. Pollen sterility varies from 82 to 98 per cent among the diploid species and ranges from 71 to 89 per cent among the autotetraploids.

4. An extensive cytological survey of the species allied to *T. virginiana* shows them to be made up largely of (1) diploid races and species, mostly southern, and (2) autotetraploid races and species, mostly northern in distribution.

5. The autotetraploids have roughly twice the average radius of distribution of the diploids.

6. Terminal chiasmata and median attachment constrictions, conditions favoring autotetraploidy, are discussed.

7. Autotetraploid Tradescantias are slightly larger and have longer blooming periods than the related diploids, but can be distinguished from them only cytologically.

8. Cytological conditions favoring autotetraploidy have brought about the independent evolution in different species of *Tradescantia* of vigorous autotetraploid races.

9. Five artificial interspecific diploid hybrids were produced experimentally and studied cytologically. Pairing in these hybrids is regular although the chiasma frequency is slightly lower than the average of the parents. Pollen fertility varies from 40 to 73 per cent. The evidence therefore indicates that structural changes have played little or no part in the immediate differentiation of these species.

10. Natural hybrids are discussed and illustrated by examples.

11. Certain of the internal and external barriers to interspecific hybridization in the American Tradescantias are enumerated.

12. The various consequences of hybridization under different conditions are described.

13. The cultivated Tradescantias are compared with the wild species from which they were ultimately derived and their cytological differences are compared statistically.

Conclusions

Evolutionary processes in the Commelinaceae

Structural change has evidently been an important factor in the differentiation of genera. Polyploidy (allopolyploidy, autopolyploidy, aneuploidy), segmental interchange, fragmentation, and differences in chromosome size are the most conspicuous changes which have occurred.

Evolutionary processes in T. virginiana and its relatives

1. A special feature of evolution in this group is autopolyploidy. Rare (or at least seldom reported) in other strictly wild species, it is here the rule. Conditions favoring its development have encouraged the independent evolution in the whole group of species, of vigorous autotetraploid races. Autotetraploidy, although it complicates the relationships between species, and although it enriches the pattern of variation within species, cannot be considered a factor of major evolutionary importance, even in these Tradescantias.

2. Factors of general evolutionary importance here are structural changes, hybridization, and genic differentiation:

(*a*) *Structural changes.*—Various examples were occasionally found, as for instance, fragmentation and chromosome interchange. There was, however, no evidence that these factors had been of any importance in the immediate differentiation of these species.

(*b*) *Hybridization.*—It is in *Tradescantia* an evolutionary feature of particular importance, deserving of careful field analysis. Its effects are various, depending upon both internal and external variables.

(*c*) *Genic differentiation.*—Apparently the major factor in the differentiation of these species has been the slow accumulation of genic differences. As to when, where, or how these hypothetical genic differences have accumulated we have as yet no evidence. For the present we can only dignify our ignorance with a scientific term and conclude that "genic differences" have been the most important factor in the immediate development of these American Tradescantias.

The writers have carried out the work reported here while serving as members of the staff of the Arnold Arboretum of Harvard Uni-

versity. The actual growing of the living material and its cytological examination have taken place at their alma mater, the Bussey Institution of Harvard University. For the privilege of sharing in the stimulating atmosphere and material advantages of this latter laboratory of which they are staff members only by courtesy, they are profoundly grateful.

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EFFECTS OF NUTRIENT CONCENTRATION ON ANATOMY, METABOLISM, AND BUD ABSCISSION OF SWEET PEA

G. T. NIGHTINGALE AND R. B. FARNHAM

(WITH TWENTY-ONE FIGURES)

Introduction

For several years the writers have been conducting experiments concerned with the premature abscission of flower buds of sweet pea, *Pisum lathyrus odoratus*. Under commercial greenhouse conditions the sweet pea is often grown in a fertile soil high in organic matter and plentifully supplied with moisture. Under such treatment, a vigorous succulent growth usually occurs, accompanied, in the short cloudy days of winter, by abscission of a high percentage of the buds. Experimentally plants have been grown under different environmental conditions and with varied nutrient media, but throughout the course of the work one fact stood out clearly: abscission of the buds was intimately associated with the character of growth of the plants. It occurred to the greatest extent on plants that grew vigorously, were comparatively succulent, and had thin dark green leaves of relatively large area.

Chemical analyses of such plants showed that they were invariably high in percentage concentration of amino acids and proteins but low in reserve carbohydrates. Limitation of the supply of available nitrogenous nutrient seemed therefore to be indicated as a probable means by which plants having a lower concentration of organic nitrogen and a higher concentration of carbohydrates could be grown less vigorously vegetative. A low plane of nitrogen supply in sand culture tended to give this result and also a decreased percentage of bud drop. In the case of sweet peas under commercial conditions, however, when the daily and seasonal variations in sunlight and, therefore, in rate of carbohydrate synthesis were very great, it was found difficult on the one hand to avoid extreme nitrogen deficiency and carbohydrate accumulation resulting in a small yield of flowers

with short peduncles; on the other hand it was found equally difficult to avoid excessive vegetative growth, which was associated with abscission of buds and a high percentage of elaborated nitrogen in the tissues of the plant but a low concentration of carbohydrates.

Observations of the practices of a few growers who were more successful than the average in controlling abscission of flower buds indicated that in general they employed soils which were high in concentration of plant nutrients, including nitrate. During the winter period, however, when photosynthetic activity was limited by relatively little sunlight, they watered the plants sparingly although the plants were not permitted to wilt. Under such treatment there was produced a moderately vigorous vegetative growth that was not excessively succulent, fewer flower buds abscissed, and a good yield of flowers was obtained. The plants did not seem to be materially limited in their capacity to absorb nutrients because of the low supply of water in the soil. Nitrate and mineral nutrients occurred in abundance in the tissues of roots and tops. Nevertheless, the plants were not high in concentration of the elaborated forms of nitrogen such as amino acids and proteins, but did contain a relatively high percentage of reserve carbohydrates.

Assuming that one of the principal effects of a low soil moisture content was an increase in the concentration of salts in the soil solution, tests in sand culture were made. High and low concentrations of a complete nutrient solution containing abundant nitrate were employed. The results of these experiments are discussed in the following pages.

Experimental methods

On August 4, 1934, seeds of the variety Balls Rose were planted in white quartz sand in 12-liter self-draining porcelain jars. A few days after the tops emerged above the sand the plants were selected for uniformity and were thinned to two plants per jar. All received the nutrient solution indicated in table I, made up with tap water at an approximate concentration of one half atmosphere. One liter of solution was supplied as one application per day to each culture. After September 30, the solution was percolated through the sand according to the constant renewal method of SHIVE and STAHL (31), and the amount of solution was increased to 2 liters per culture per

24 hours. Also at that time the plants were divided into four groups of thirty-two plants each, each group receiving the same proportion of nutrient salts but at approximate concentrations of one half, one, two, and three atmospheres respectively. On November 1, the amount of solution percolated through the sand was increased to 3 liters per culture per day.

TABLE I

COMPOSITION OF NUTRIENT SOLUTIONS

Approximate concentration of nutrient solutions in atmospheres	Partial volume molecular concentration of salts employed		
	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$
½	0.0022	0.0045	0.0022
1	0.0045	0.0090	0.0045
2	0.0090	0.0180	0.0090
3	0.0135	0.0270	0.0135

The pH estimations of nutrient solutions were made colorimetrically before and after passing through the sand. As applied, the solutions (adjusted with KOH) were approximately pH 5.6 but after passing through the sand they frequently increased in acidity to pH 4.8.[1] The effect of such increase in acidity was minimized by frequent flushing of the cultures with 0.02 normal KOH, followed immediately with fresh nutrient solution. The pH range of the cultures from 5.6 to 4.8 is such, however, as to permit good growth and assimilation of nitrate (**13, 19, 32**) if other environmental conditions are favorable.

Aside from the sand culture treatments, the usual commercial procedure was followed in growing the plants. In so far as seasonal conditions permitted, the air temperature of the greenhouse was maintained at approximately 45° F. at night, during the day at about 50° if the weather was cloudy, and at 60° to 65° F. on bright sunny days. The relative humidity of the air was kept at about 75 to 85 per cent, preferably at the higher figure.

[1] When the sole external source of nitrogen is from a nitrate salt, as in the case of these experiments (**19**), cultures of non-leguminous plants commonly increase in alkalinity rather than acidity.

Boron, manganese, and iron were apparently present in sufficient quantity in the salts employed or in the tap water. At least additions of these elements to some of the cultures of each series produced no noticeable effect.

The plants were divided for macroanalysis into (1) leaf blades, (2) stems and petioles, and (3) roots, the last fraction including also such portion of the stem as occurred beneath the surface of the sand.

Determinations of nitrogenous and carbohydrate fractions were made with fresh and dried tissue respectively according to methods recently described (**4, 22**). ECKERSON'S (**10**) microchemical technique was followed and her procedure was employed in making reducase determinations (**7**).

In the preparation of permanent slides, well recognized methods were followed. Plant material was fixed in Navashin's solution, dehydrated, imbedded in paraffin, sectioned and stained with safranin and gentian violet. All drawings were made on the same scale with the aid of a projectoscope.

Records of yields and quality of flowers were taken and will be summarized in the following pages, but the junior writer will report the detailed results in another publication concerned with the use of different nutrient media and the direct commercial application of the principles which are emphasized in the following discussions.

Results

EXTERNAL APPEARANCE

The obvious effects on the plants of the respective concentrations of nutrient salts became evident rather gradually following the shift in treatments on September 30. After a week or ten days there was a noticeable effect on color of foliage, which in two or three weeks was followed by distinct differences in character of growth of all organs of the plant. Comparative differences in volume of growth as they occurred on December 9 are given in table II, and photographs taken a few days later are shown in figures 1, 2, and 3.

The plants grown in nutrient solutions of concentrations of one half and one atmosphere, respectively, were very similar in volume and quality of growth. Plants in the higher concentrations of two and three atmospheres were also similar to each other (table II, figs.

1, 2). For convenience the similar groups will be described together. Those at one half and at one atmosphere are designated as low concentration cultures and the plants at two and at three atmospheres as high concentration cultures.

ROOTS.—Figure 1 shows a typical pair of root systems from a single culture jar of the low and from a single culture jar of the high concentration groups. This illustration together with the data of table II shows clearly that there was a much greater volume of roots produced at low than at high concentration of nutrients. Of equal

TABLE II

COMPARATIVE AVERAGE GROWTH PER PLANT OF SWEET PEA; DECEMBER 9, 1934

	Approximate concentration of nutrient solution in atmospheres			
	½	1	2	3
Total green weight per plant				
Blades (gm.)	82	99	76	67
Main stem plus lateral stems plus petioles (gm.)	166	170	101	99
Roots (gm.)	66	57	40	38
Total linear growth per plant				
Main stem plus lateral stems (cm.)	1400	1400	1000	950

significance is the character or quality of the roots produced. At the lower two concentrations the roots were glistening white and typically of larger diameter. Except in the case of the two or three main roots the cortex was obviously alive during the whole period of the experiments. The roots were not brown even at their older or proximal ends. They were highly succulent and lacking in mechanical strength.

In contrast, the roots of the high concentration cultures were typically woody, mechanically strong, and distinctly lacking in succulence except near the distal end. Early maturity of the cortex was not due to plasmolysis of the cortical cells by the concentration of nutrient salts employed. Root hairs were present in abundance and showed no indication of plasmolysis, although they persisted for only a short time because of the rapid loss of cortical tissue.

The quality of growth of sweet pea roots at high salt concentrations was very similar to that exhibited by the roots of apple and peach trees, which were grown with the same kind of nutrient solution used here for sweet pea at a concentration of one atmosphere but at a temperature of 75° F. and higher. Likewise, the effects of a low concentration of nutrients on sweet pea roots were essentially similar

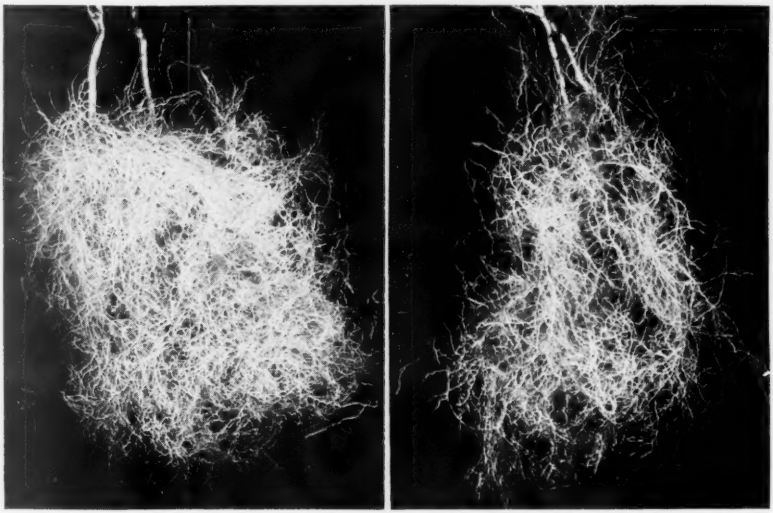


FIG. 1.—Sweet pea roots Dec. 9. Pair of root systems (left) supplied with dilute nutrient solution (one half atmosphere). Note roots are white and that secondary laterals are of relatively large diameter. They were succulent and matured slowly. Pair of comparable root systems (right) from single culture jar supplied with concentrated nutrient solution (three atmospheres). Note smaller volume of growth, that roots are not white, and that secondary laterals are of small diameter. They were relatively woody and matured rapidly.

to the effects on apple and peach tree roots of temperatures of 65° F. and lower (**20**). In both cases the tops of the peach and apple trees referred to were exposed to an air temperature of 65° F. In other publications there are discussed effects of air temperature on the growth of the tops of plants (**18, 22, 23**).

STEMS.—The effects of the respective nutrient treatments on the relative amount of leaf and stem growth are indicated in table II and

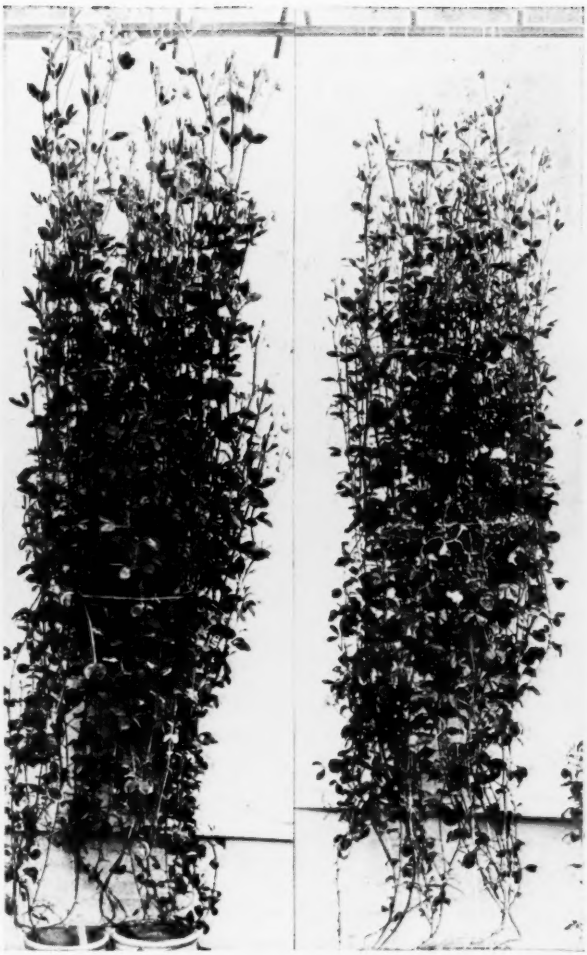


FIG. 2.—Tops of sweet pea plants Dec. 9. Four plants (left) grown two to a culture jar supplied with dilute nutrient solution (one half atmosphere). These plants grew vigorously, were dark green, comparatively soft and succulent, and many flower buds abscissed. Comparable group of plants (right) grown in concentrated nutrient solution (three atmospheres). Note smaller volume of growth. These plants were lacking in succulence, light green, and relatively few flower buds abscissed.

FIG. 3.—Leaves (left) typical of plants supplied with dilute nutrient solution (one half atmosphere). Note comparatively large area and nearly circular shape. Blades were thin, succulent, and dark green. Leaves (right) typical of plants supplied with concentrated nutrient solution (three atmospheres). Note small area and elliptical shape. They were thick, relatively less succulent, and yellowish green.

Flowers and buds (left) from plants grown in dilute nutrient solution. Peduncles soft and succulent, straightening prematurely, accompanying which buds became yellowish and abscissed. Buds and flowers (right) from plants grown in concentrated nutrient solution. Peduncles shorter, less succulent, remaining downwardly curved until full bloom. Comparatively few flower buds abscissed.

figure 2. The quality of growth and yield of flowers were definitely influenced by the concentration of salts employed. The greater total linear growth of stems at the lower concentrations was due principally to the development of many lateral shoots. The stems of the plants grown at low concentrations were also much larger in diameter than at the higher concentrations, much more succulent, the internodes were longer, and the winglike lateral expansions of the stem were darker green and very large, making the stem appear relatively flat. The high concentration cultures had stems of lighter green, which were typically rather woody except near the tips, and the lateral expansions were smaller and persisted for a shorter time. The stems were accordingly more or less circular in cross section except near the distal end.

LEAVES.—The differences in amount and character of the foliage of the respective groups were also striking. The total green weight of the blades was much greater at one half or one atmosphere than at the higher concentrations (table II), but perhaps of even greater significance was the marked difference in quality of the leaves. The blades of the low concentration cultures were dark green, nearly circular in outline (fig. 3), very thin, extremely succulent, and they wilted almost immediately after picking. The petioles and tendrils were likewise succulent, relatively long, and of comparatively large diameter. The latter were frequently more or less foliate. In contrast, the blades of the plants of the high concentration cultures were typically lighter green, much thicker, almost leathery to the touch, smaller in area, and oval to eliptical in shape (fig. 3). Even an hour or more after picking and in the absence of an external supply of water, the leaves showed little evidence of wilting. Coupled with these properties the development of tendrils was much less extensive, they often exhibited a reddish tinge, and they, as well as the comparatively short petioles, were of small diameter and lacking in succulence.

These characteristics of leaves and stems are not peculiar to the effects of the concentration of salts in the nutrient medium, nor are they limited to the sweet pea. Some of the many factors (**15, 18, 22, 23, 24, 27, 28**) which have directly or indirectly resulted in similar responses in other plants will be discussed in the following pages. It

may be pertinent, however, to point out that in case of apple, BLAKE and DAVIDSON (1) have found that with varying degrees of vegetative vigor and fruitfulness there was variation in shape of the spur leaves, from more or less circular in case of the strongly vegetative type to oval or eliptical in case of the leaves borne on spurs that were typically less vigorously vegetative and characteristically produced fruits of high color for the variety. These differences in shape of the leaves are therefore very similar to those recorded for sweet pea, differences which in apple furnish for fruit growers an index of the degree of vegetative and reproductive vigor of their trees.

TABLE III

ABSCISSION OF BUDS AND LENGTH OF PEDUNCLES OF SWEET PEA

ATMOSPHERES	APPROXIMATE CONCENTRATION OF NUTRIENT SOLUTION					
	PERCENTAGE OF BUDS THAT ABSCISSED			AVERAGE LENGTH (CM.) OF PEDUNCLES*		
	NOVEMBER	DECEMBER	JANUARY	NOVEMBER	DECEMBER	JANUARY
½	46	59	31	26	23	23
1	33	47	26	26	20	23
2	12	30	7	25	22	16
3	10	24	3	24	20	15

* Peduncles from buds that abscissed are not included in averages; as a rule they were 5 to 6 cm. longer than the average.

FLOWERS.—Associated with the vegetative responses already described there occurred in the low concentration cultures a much higher percentage of blossom bud abscission than in those of high concentration (table III, fig. 3). After a few days of cloudy weather the developing vegetative growth of the plants supplied with the low concentrations of nutrients always became extremely soft and there was frequently nearly 100 per cent loss of blossom buds. This was demonstrated repeatedly, and often a week or more before the flower buds actually abscissed the peduncle would straighten (fig. 3) instead of remaining in the usual downwardly curved position. Accompanying this response, the buds gradually changed from dark to yellowish green. Extremely soft, succulent growth of the vegetative organs

was invariably accompanied by the development of soft succulent peduncles and the straightening and change in color described, and by an increase in percentage of abscission.

In general the peduncles in this series were relatively long, especially in the case of those buds which abscissed. Those flowers which persisted were borne on the shorter peduncles (table III).

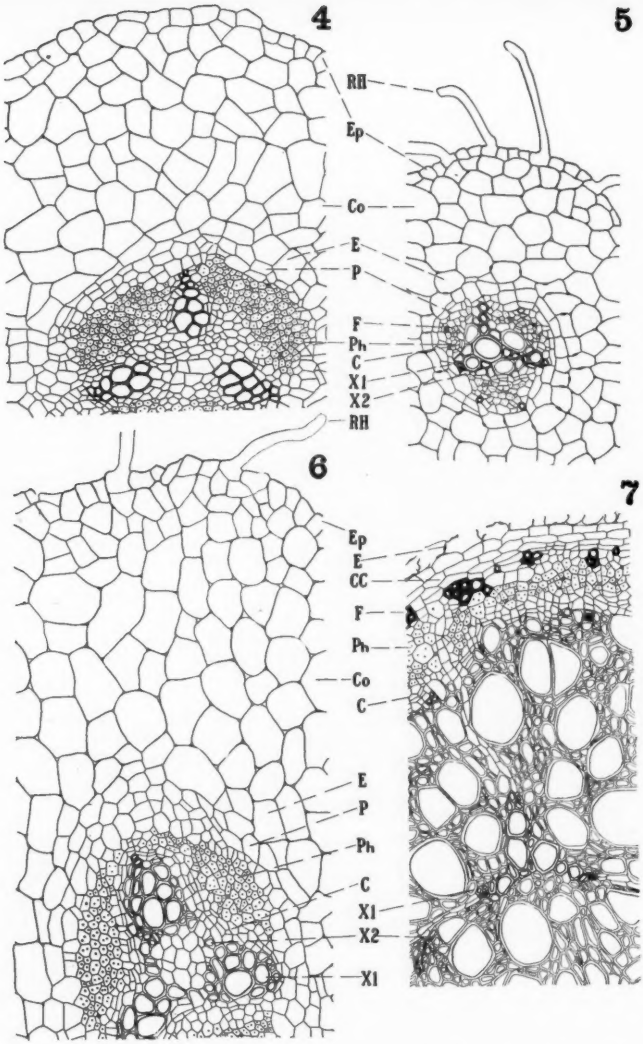
The less succulent plants of the high concentration cultures were correspondingly low in percentage loss of flower buds and had peduncles which remained downwardly curved, were less succulent, of smaller diameter, and shorter. Also the flowers from these non-succulent plants were much superior in keeping quality to those borne by the more succulent plants. Cultures of the latter group produced 35 per cent fewer marketable inflorescences, although when the buds persisted there were, as a rule, a larger number of individual flowers per inflorescence.

Anatomical structure

In anatomical structure, as well as in external appearance, the plants of the groups at one half and one atmospheres were very similar, as were those at two and three atmospheres. It will be unnecessary, therefore, to discuss separately the plants of the four different nutrient treatments (table I). The anatomical drawings described were made from representative plants of cultures which were supplied with the nutrient solution at a concentration of one half atmosphere and three atmospheres, respectively.

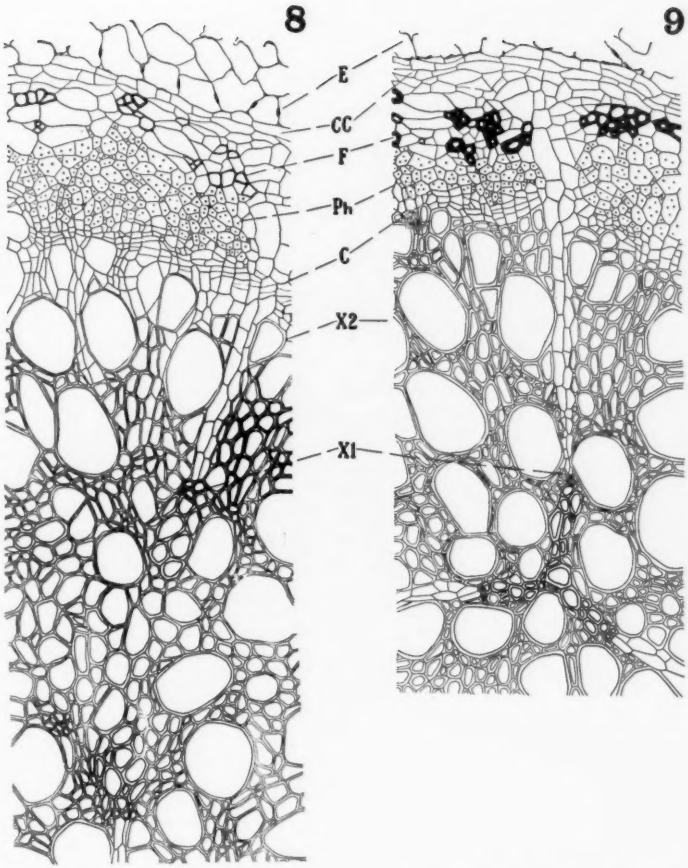
Roots.—It has already been recorded that the plants which received the lower concentrations of nutrient salts produced root systems that remained white and relatively succulent for the entire duration of these experiments (fig. 1), whereas at higher concentrations the cortex rapidly became brown and the central cylinder tough and woody. The associated anatomical situation is shown in figures 4–10.

It is apparent that the rate of maturity of tissues was much slower at low than at high concentrations of nutrient salts. At 1 cm. from the root tip developed in low concentration solutions there were practically no root hairs, the cortical cells were comparatively angular, closely packed, and Casparian strips were not yet noticeable



FIGS. 4–7.*—Transverse sections of sweet pea roots: fig. 4, 1 cm. from tip, dilute nutrient solution; fig. 5, 1 cm. from tip, concentrated nutrient solution; fig. 6, 5 cm. from tip, dilute nutrient solution; fig. 7, 5 cm. from tip, concentrated nutrient solution. Note early differentiation and maturity of tissues (figs. 5, 7); cortex sloughed off (fig. 7).

* Abbreviations for figs. 4–21: *Ep*, epidermis; *Co*, cortex; *E*, endodermis; *CC*, cork cambium; *P*, pericycle; *F*, fibers; *Ph*, phloem; *C*, cambium; *X1*, primary xylem; *X2*, secondary xylem; *Pi*, pith; *M*, mesophyll; *S*, stoma; *UEp*, upper epidermis; *LEp*, lower epidermis; *AZ*, abscission zone.



FIGS. 8, 9.—Transverse sections of sweet pea roots 15 cm. from tip: fig. 8, dilute nutrient solution (cortex alive but not shown); fig. 9, concentrated nutrient solution (cortex sloughed off heavy fibers and few cells in cambium region).

in the endodermis (fig. 4). In the central cylinder there were at low concentration relatively many cells of primary phloem and xylem but definite lignification was apparent only in the region of the protoxylem. The remainder of the stele consisted largely of small, closely packed, thin walled cells containing abundant protoplasm (fig. 4).

At low concentrations of nutrient at a point 5 cm. from the root tip (fig. 6) there were many root hairs, the cortical cells were somewhat more rounded and larger than at the earlier stage of development shown in figure 4, but there was no indication of death of the cortex. The central cylinder as shown in figure 6 (at 5 cm. from the root tip) was remarkable in that progress toward maturity had proceeded so slowly. Noticeable Casparian strips were lacking, there was not the slightest indication of fibers, and there was little lignified xylem. Many cells even of the metaxylem were still thin walled and, similar to the cells of the phloem and pericycle region, had rather dense protoplasmic contents.

The roots of the low concentration cultures at 15 cm. from the root tip exhibited in the region of the stelar cambium a remarkably wide band of thin walled cells containing relatively dense protoplasm (fig. 8). It will be recalled that, at the earlier stages described (figs. 4, 6), the growth of the roots at low concentrations included primary tissues that were slow in maturing, but practically no secondary elements. Nevertheless, at 15 cm. from the root tip there was an abundance of secondary phloem and xylem both of which matured slowly. At this stage the cortex, only a part of which is shown in figure 8, was sometimes more or less torn internally, although it was apparently alive and in external appearance smooth and white. The endodermis was usually intact and Casparian strips were clearly evident. Underlying the endodermis, there was some indication of a potential cork cambium, although during the period of the experiments there was practically no suberization of cell walls in that region and but comparatively slight thickening of the walls of the fibers.

In contrast, the plants receiving the higher concentrations of nutrient solution at 1 cm. from the root tip had few cortical cells. They were small and obviously rather mature in that they were rounded

and deficient in protoplasmic contents (fig. 5). Many root hairs were in evidence in this region, as were distinct Casparian strips. The elements composing the small central cylinder of the roots which developed at higher nutrient concentrations (fig. 5) were also relatively mature. There were even present a few thick walled fibers over the primary phloem. All the elements of the primary xylem were strongly lignified and optically empty. There were a few secondary xylem and phloem elements owing to the early initiation of cambial activity. The secondary tissues of xylem and phloem, however, were extremely limited in amount and matured rapidly.

The roots of the high concentration cultures (fig. 7) had lost practically all cortical tissue at 5 cm. from the root tip, and from the region of the pericycle had arisen a cork cambium some of whose external derivatives had become strongly suberized. The roots were therefore rough and brown in external appearance as compared with those of the dilute nutrient solutions. Together with loss of the cortex and the appearance of a strong cork cambium, there were present many heavy walled fibers, and the bulk of the stele consisted of strongly lignified, optically empty secondary xylem elements. Most of the phloem tissue was also comparatively mature, and while the cambium was obviously active in the production of secondary tissue, the cells derived from it matured with extraordinary rapidity, leaving in consequence only a limited amount of embryonic tissue in the cambium region.

The roots which were produced in the cultures of high concentration presented much the same situation at 15 as at 5 cm. from the root tip (fig. 9). Fibers were somewhat thicker walled and there was a small increase in volume of secondary xylem and phloem, but the general characteristics were as already described.

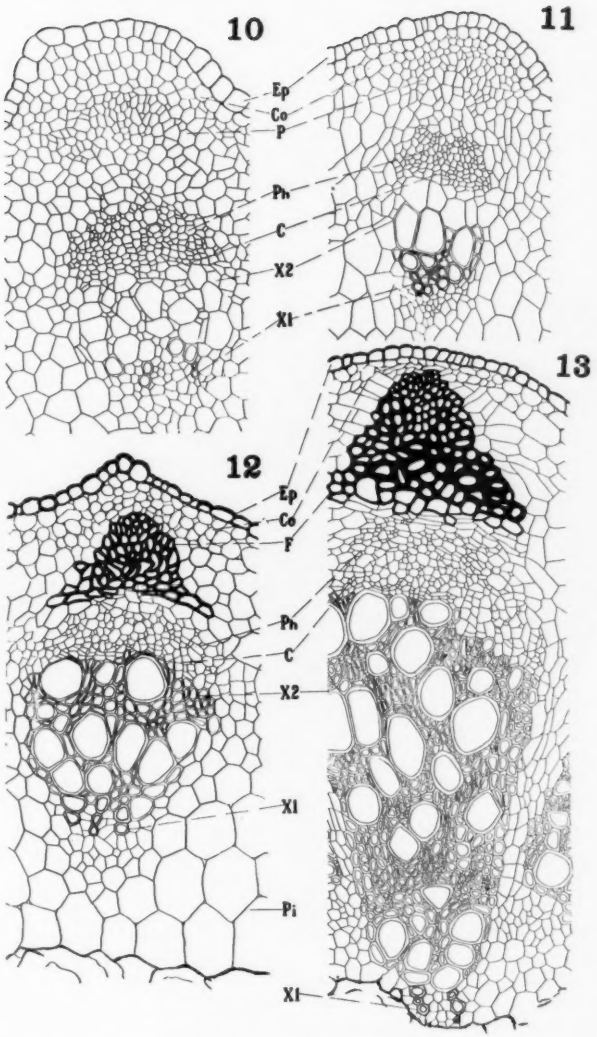
The roots of the high concentration cultures have been characterized as woody, mechanically strong, and lacking in succulence. Associated with these conditions the well known fact may be recalled (16) that, although the intake of nutrients may occur freely with a fairly high concentration of salts in the nutrient solution (table V), there is a marked diminution in absorption of water. This factor undoubtedly contributed in part to the early loss of protoplasm and the rapid maturity of tissues when sweet pea was grown in a nutrient

medium of two or three atmospheres. This is in contrast with the comparatively slow maturation of the roots of the low concentration cultures.

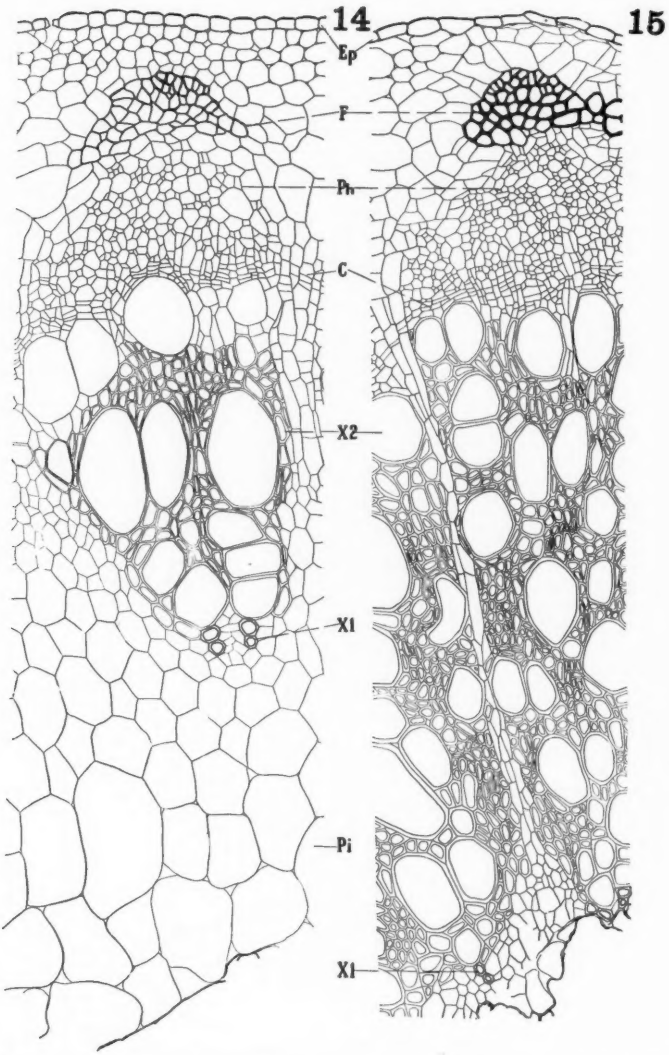
It should be emphasized, however, that factors other than salt concentration may give very similar responses. For example, the anatomical responses of the roots of peach and apple (**20**) which were grown at a temperature of 75° F. (with the same nutrient solution as in these experiments, at a concentration of one atmosphere) corresponded in almost every detail with that of sweet peas as grown at two or three atmospheres at about 50° F. That the metabolic responses were also much the same will be shown later. Likewise peach and apple (**20**), with the nutrient treatment described above but at a temperature of 65° F., exhibited roots which resembled in anatomy and metabolism the roots of sweet pea which were supplied with the more dilute nutrient solutions.

STEMS.—In the preceding pages it has been shown that the lower concentrations of nutrient salts directly or indirectly resulted in the production of vigorously growing stems of comparatively large diameter; whereas with more concentrated solutions the stems grew less vigorously, were smaller in diameter, and comparatively woody (table II, fig. 2). The associated anatomical features are indicated in figures 10 to 15.

Even casual examination of these figures shows that the structure of the stems was influenced by the concentration of nutrient salts in a manner similar to that already recorded for the roots of sweet pea. It is obvious that the rate of maturity of tissues was much slower at low than at high concentrations of nutrient solution. At 1 cm. from the stem tip the plants of the more dilute solutions (fig. 10) exhibited in transverse section many cells which were practically all thin walled, closely packed, and filled with dense protoplasmic contents. Lignification was evident only in a few cells of the protoxylem, the metaxylem was extensive but thin walled, and there was only slight indication of cambial activity. In contrast, the stems of the cultures supplied with the more concentrated solution had at the same distance from the stem tip (fig. 11) much less primary tissue; and the cells, except in the region of the cambium and pericycle, were comparatively limited in content of opaque protoplasm. Further, the



FIGS. 10–13.—Transverse sections of sweet pea stems: fig. 10, 1 cm. from tip, dilute nutrient solution; fig. 11, 1 cm. from tip, concentrated nutrient solution (note fewer primary cells and earlier differentiation); figs. 12, 13, concentrated nutrient solution, middle and base of stem respectively.



FIGS. 14, 15.—Transverse sections of sweet pea stems, dilute nutrient solution middle and base of stem respectively. Compare with figures 12 and 13 which show earlier maturity of tissues, heavier fibers, and limited cambium region with concentrated nutrient solution.

primary vascular elements, although much less in total volume than at lower concentration of nutrients, were strongly lignified and optically empty in both protoxylem and metaxylem regions, and the cells of the phloem were comparatively limited in content of protoplasm. Likewise in accord with the rapid differentiation of primary tissues there was apparently present some secondary phloem and xylem, the latter rapidly undergoing the early stages of lignification and loss of cell contents.

Transverse sections of typical vascular bundles (figs. 12, 14) at about the middle of the respective primary stems yielded information strictly in harmony with the preceding observations. The plants supplied with the higher concentrations of nutrient salts showed that there had been not only more rapid maturity of primary elements, including loss of much of the pith, but that there was rapid maturity of secondary tissues as well. Associated with the more dilute nutrient solutions many more pith cells persisted and exhibited protoplasmic contents, and there was much more extensive development of secondary xylem and phloem, the cell walls of the former becoming lignified and losing their cell contents slowly, even the oldest cells of the phloem showing denser protoplasm. The slow differentiation of secondary elements necessarily left in the cambium region a remarkably wide band of young, thin walled cells (fig. 14). In the region of the pericycle there were in evidence only the early stages of development of fibers.

Fibers of the groups at high concentration of nutrients, however, were very thick walled (fig. 12), the cortical cells more rounded, and the epidermal cells and cuticle considerably thickened. Secondary phloem and xylem matured so rapidly that there was in transverse section but a narrow band of cells in the cambium region. All these factors contributed to the relatively small diameter and lack of succulence of the stems of the sweet pea plants which were supplied with the higher concentrations of nutrient solution.

Drawings were also made (figs. 13, 15) of transverse sections of the base of the stem just above the transition region of exarch and endarch xylem (13). The stems at this point presented a situation in accord with that exhibited by the middle of the stem. Owing to a very active cambium there was much more secondary xylem and phloem

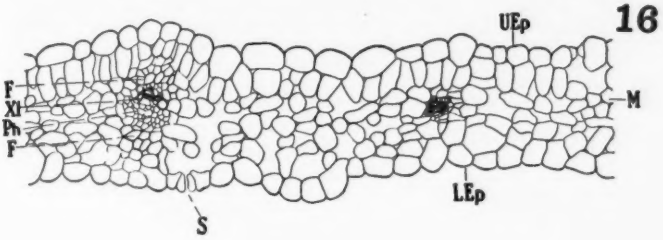
and a correspondingly thicker stem at low than at high concentrations of nutrient, but pericycle fibers in the former were limited in number and in thickness, and in the region of the cambium there were many actively dividing cells containing dense protoplasm. In clear cut contrast, the base of the stems of the cultures of higher salt concentration (fig. 13) showed large bundles of strongly developed pericycle fibers, and phloem and xylem which were much less extensive owing to less cambial activity. In fact in the entire transverse section there were few cells that were not optically empty and, in the region of the cambium, mature phloem and strongly lignified xylem elements frequently abutted, one upon the other.

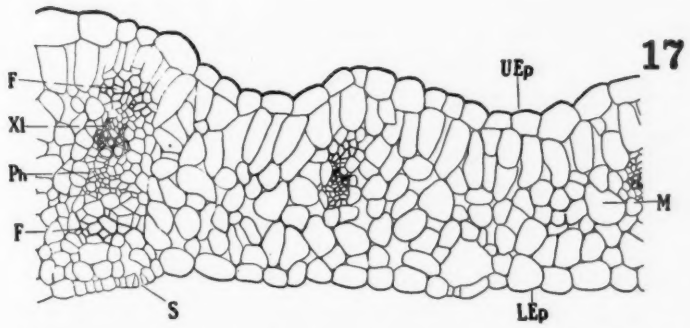
It is obvious that these anatomical features were intimately associated with the external growth responses already recorded, the condition of woodiness and lack of succulence at higher concentration of nutrients, and the comparatively soft and extremely succulent growth of stems of the plants supplied with the more dilute solution of nutrient salts.

LEAVES.—The anatomical structure of the thin, dark green, succulent leaves of the plants supplied with dilute nutrient solutions is indicated in figure 16. This may be compared with that of the cultures of higher nutrient concentrations (fig. 17) which, it will be recalled, bore leaves that were smaller in area, lighter green, thicker, and much less succulent (fig. 3). The associated anatomical differences are clearly in harmony with the external appearance of these leaves.

At high concentration of nutrients the cuticle of the upper epidermis was heavy, the palisade mesophyll distinct, and the spongy mesophyll relatively compact. In contrast, the plants supplied with more dilute nutrients exhibited leaves in which the spongy mesophyll was prevalent and loosely arranged, in that there were larger and more numerous intercellular spaces. The somewhat smaller size of mesophyll cells in the latter group, however, is at variance with the relative size of cells in roots or stems of corresponding series. It will be remembered that these organs had cells which were notably large in the plants of the low as compared with those of the high concentration cultures. Why the blades failed to present a corresponding situation is not certain. It may be mentioned, however, that the per-

centage of sugars and starch was extremely low in the blades of the plants which received the more dilute nutrient solutions (table V). The low content of sugars may have been intimately associated with the osmotic concentration of the cells concerned and consequently with cell expansion. In the stems and roots of the same group of





FIGS. 16, 17.—Transverse sections of sweet pea leaves, dilute and concentrated nutrient solution respectively. Note relatively xerophytic structure of leaf from plant supplied with concentrated nutrient solution.

plants, correlated with a high water content but less drastic reduction in percentage of sugars and starch, there was found the comparatively large size of cells and slow rate of maturation of tissues already mentioned. It should be emphasized, however, that, as in other organs of the plants, the blades of the cultures supplied with the more dilute solution of nutrient salts appeared much less vacuolated and contained larger and apparently darker green chloroplasts

than the plants which received the more concentrated nutrient solutions.

Between the two groups there was little if any difference in the number of stomata found in corresponding areas of mature leaves. Repeated measurements demonstrated, however, that the veins were much more numerous per unit area in the blades of the high concentration cultures; further, the bundles of fibers of the veins were larger and thicker walled (fig. 17). Frequently veinlike bundles were found in which only fibers were clearly evident.

The preceding descriptions are obviously not specific as to effects of the respective salt concentrations of the nutrient medium. The blades of the high concentration groups are similar in external appearance and anatomy to so-called "sun leaves" or to leaves which develop under xerophytic conditions including a limited supply of soil or atmospheric moisture or both (**16, 24**). Nevertheless such a response would be anticipated, as it is well known that with increase in concentration of salts in the nutrient or soil solution there is a marked diminution in rate of water absorption (**16**). Conversely with a more dilute solution of salts there is greater intake of water. The blades of the sweet pea plants with the less concentrated nutrient solutions exhibited the typical characteristics of plants in a fertile soil with abundant moisture, or of plants grown at high humidity or in the shade where likewise the percentage of dry matter in the plant is low but the percentage of water high (**15, 16, 24**).

ABSCISSION ZONE.—The structure of the peduncle and pedicel of sweet pea was found to be similar to that of the stem except that cambial activity apparently ceased at about the time buds abscissed or blossoms matured. The anatomical structure of the flower stalk and pedicels was affected by the concentrations of nutrient salts employed in essentially the same way as were the stems. It will therefore be unnecessary to present a detailed anatomical description of these plant parts.

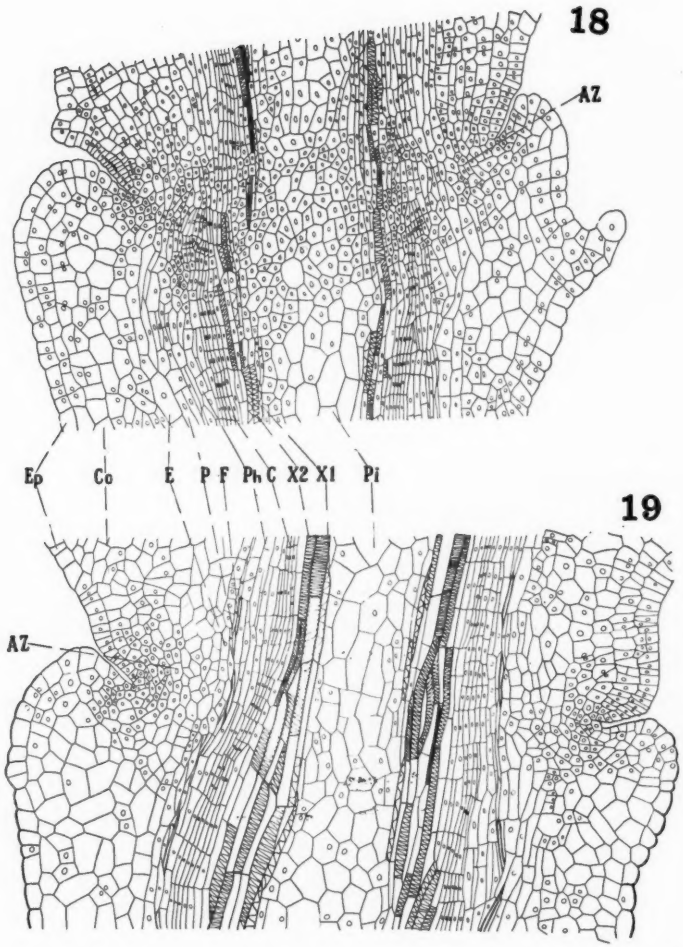
As shown in table II, in neither nutrient series did all of the flower buds absciss. At low concentration of salts, these peduncles that bore flowers to maturity were of comparatively large diameter and succulent, owing, as in case of stems of the same group, to relatively many primary cells, greater cambial activity, and larger cells of both

primary and secondary tissues, both of which differentiated and matured slowly. Following or accompanying a period of cloudy dark days, however, the peduncles and pedicels became more succulent, maturation became even slower, and from the plants of the low concentration cultures there was frequently nearly 100 per cent abscission of buds, separation occurring at the point of attachment of the pedicel to the main flower stalk.

At this point, the peduncle and pedicel of such abscissing buds were typically of small diameter, as illustrated in figure 18, which shows an approximately median longitudinal section through the potential abscission zone eight days after the appearance of a bud. This bud, typical of many others of the same group of cultures, was obviously going to absciss as evidenced by the premature straightening of the peduncle (fig. 3). It was borne on a pedicel remarkable for its immaturity. With the exception of a few primary xylem vessels, there was no evidence of lignification and fibers were absent. Throughout much of the pedicel, even in cortex and pith, dense cytoplasm and large nuclei prevailed together with a high concentration of amino acids and proteins.

In the region of the potential abscission zone at the approximate level of the grooved ring of the cortex (fig. 18), there was clearly evident tissue ten to fifteen cells in thickness that was delimited by the smaller size of its cells and their very dense contents. It extended transversely through the pedicel, and with the exception of the primary xylem vessels practically all cells at this level exhibited the condition described. At this stage the cells of this potential abscission layer were not noticeably different from neighboring cells, above and below, in content of mineral nutrients. Reducing sugars and sucrose were present but practically no starch. As already mentioned, the protoplasmic content of the pedicels was high and particularly so in the cells of the zone of abscission. After denaturization with dilute alkali, the contents of such cells gave strong protein reactions.

The material for figure 19 was obtained from a plant of the series which received the highest concentration of nutrient salts (table I) and from a pedicel typical of many others of that group of plants. The pedicel was bearing a bud which presumably would not have ab-



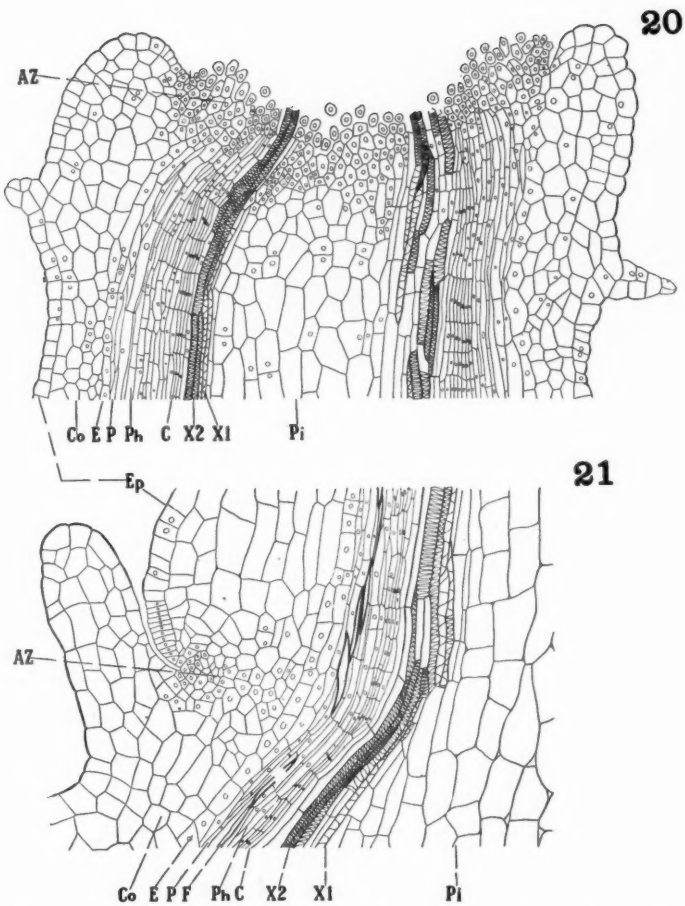
FIGS. 18, 19.—Longitudinal sections through pedicel and peduncle of sweet pea in region of abscission zone 8 days after bud was evident, dilute and concentrated nutrient solution respectively. Note that with dilute nutrient solution differentiation and maturity of tissues were slow. Several tiers of meristematic cells in region of potential abscission zone (fig. 18).

scissed, as indicated by the condition of the peduncle which was downwardly curved (fig. 3). Further, the peduncle and pedicel were relatively woody and lacking in succulence.

Figures 18 and 19 are comparable in the sense that both drawings were made from material harvested eight days after a bud had appeared on the pedicel concerned. However, the similarity ends there. The material, although comparable in age as expressed in days, was strikingly dissimilar in degree of maturity. The peduncles and pedicels of the cultures of high nutrient concentration were much more mature in every way. The pith cells, although not yet fully enlarged, were comparatively free of protoplasm; both primary and secondary xylem were strongly lignified, and there was relatively much of the latter due to early production of secondary tissues. Sieve and companion cells were clearly differentiated, and bordering these elements there were present thick walled fibers in the region of the pericycle. In the cortex, except near the potential zone of abscission (cortical groove or junction point of peduncle and pedicel, figure 19), all cells were much vacuolated and largely devoid of nuclei. Sugars and starch were, however, notably high in all parenchymatous cells.

Four days later, comparable buds borne on the plants supplied with the dilute nutrient solution had abscissed. Immediately after abscission, the separation layer and remaining peduncle exhibited the situation indicated in figure 20. The cells of the separation layer failed to mature and showed all the characteristics of immature cells. They were comparatively small and angular and displayed dense cytoplasm and large nuclei; they contained an abundance of proteinaceous constituents, sugars, minerals, and nitrate. No deficiency of calcium in the protoplasm was noted, although this element was apparently present in large part in combination with proteins or other materials and was found in greatest abundance only following denaturization of proteins with alkali as described elsewhere (21). However, this seemed to be generally true of all meristematic tissues in sweet pea.

Accompanying foliar abscission of *Coleus*, SAMPSON (29) reported a deficiency of calcium in the cells of the separation layer and suggested that this may have an important bearing on the degree of solidity of the so-called calcium pectate middle lamella. In sweet pea,



FIGS. 20, 21.—Longitudinal sections through pedicel and peduncle of sweet pea in region of abscission zone 12 days after bud was evident, dilute and concentrated nutrient solution respectively. Fig. 20, pedicel and bud abscissed. Note cells which failed to mature in region of abscission zone. These cells became rounded and with gelatinization of middle lamella there occurred separation of pedicel from peduncle. Fig. 21, tissues mature and highly differentiated. Only half of section shown.

with the nutritional treatments here employed, there seemed to be no lack of calcium in the cells of the abscission zone nor in bordering tissues.

The actual separation of cells in the abscission layer of sweet pea pedicels was preceded by some swelling and gelatinization of the pectic constituents of the middle lamella. Although not so pronounced as in foliar abscission in *Citrus* (**14**), abscission was of the conventional type, involving dissolution of the middle lamella as described by HODGSON (**14**) and SAMPSON (**29**). The cells so freed from neighboring tissues were round, very thin walled, and seemed still alive immediately after separation from the other cells. At least they were similar in appearance to formerly adjacent cells of analogous intact tissue except that they contained starch which, at the time of their separation, was practically absent from all other cells except those of the endodermis.

Starch began to appear in the cells of the separation layer at about the time of swelling of the middle lamella. It may very well have been a secondary effect, and HODGSON, who found a similar situation in *Citrus*, suggests that "changes occur in the cell walls of the abscission zone which prevent the translocation of the products of starch hydrolysis to adjacent cells" (**14**).

Separation did not follow a right-angle plane across the main axis of the pedicel but varied apparently according to the mechanical resistance encountered. The number of tiers of cells actually taking part in abscission varied from one or two to several. Abscission usually began in the cortex in the general vicinity of the grooved ring and proceeded inward, involving all tissues except lignified xylem elements. No pericycle fibers were present in the soft succulent peduncles and pedicels of the buds which abscissed.

The peduncle below the zone of abscission (fig. 20) had matured considerably during the four-day interval, but there was nevertheless little lignified xylem and no pericycle fibers. Pith and cortical cells were angular, closely packed, and characteristically contained considerable cytoplasm and frequently nuclei.

Following abscission, the peduncle rapidly accumulated an enormously high concentration of sugars, starch, amino acids, and mineral elements, which presumably were being carried to the develop-

ing bud prior to loss of that organ. The flower stalks of inflorescences which had abscissed persisted on the plants for the duration of these experiments, and, with the accumulation of foods as already described, lost their initial succulent condition and became woody and increased somewhat in diameter.

The material for figure 21 was obtained from a culture of the series receiving a high concentration of nutrient salts and from a typical pedicel which was clearly not going to absciss and which bore a bud that was far enough advanced just to show the color of the emerging petals. The flower stalk was harvested for anatomical study twelve days after the bud first appeared, four days later than the material of the same series in figure 19. As expressed in time it was of the same age as the peduncle from which the bud abscissed (fig. 20).

There has already been described the comparatively immature succulent condition of the peduncles and pedicels of the latter group and the general process of abscission. Associated with the woody, non-succulent condition of the pedicel and persistence of buds of the former series, there was found only a few cells which were immature in character. These cells, occurring in the potential abscission zone in the vicinity of the cortical ring or groove (fig. 21), were small, angular, and thin walled, contained dense cytoplasm and large nuclei and were practically free of starch. Distal and proximal to this region the cells of the cortex were abruptly much larger, more vacuolated, and high in content of starch. Pericycle fibers were strongly developed and cambial activity gave rise to phloem and heavily lignified xylem elements. The pith cells and primary xylem parenchyma were also mature and much vacuolated, but high in concentration of starch and nitrate. Amino acids and asparagine were present in considerable quantities but were not so abundant as in case of the more succulent series of the low concentration cultures.

Abscission of the flower buds of sweet pea was therefore intimately associated with a deficiency of carbohydrates, and as usual (**15, 18, 27, 28, 34**) under such conditions tissues matured slowly and were lacking in mechanical strength. In the very young pedicels there was, of course, no apparent way of distinguishing the tiers of cells which were destined to form the abscission layer, as all tissue was

relatively meristematic. But in case of peduncles and pedicels which were low in reserve carbohydrates and obviously going to drop their buds, no subsequent or older stages could be found in which young angular cells with dense contents were not present in the region of the potential zone of abscission. On the other hand, the cultures of the series which received a high concentration of nutrient salts and which were higher in carbohydrates matured rapidly and did not exhibit this condition (figs. 20, 21).

The mode of derivation of the abscission zone already described is in contrast to the abscission through the non-succulent pedicels of mature fruits or the abscission through the comparatively woody petioles of mature leaves. For example, SAMPSON (**29**) found that in the case of the foliar abscission of *Coleus* mature cells became active and divided and thus gave rise to tiers of meristematic cells involving all tissues except the lignified xylem elements.

There have been recorded in the preceding pages effects of the salt concentrations of the nutrient solution on the anatomical structure of the vegetative organs of sweet pea. Similar effects are also described for peduncle and pedicel, together with discussions concerning the structure and composition of these organs in their relationship to premature abscission of flower buds. In the following pages it is proposed to show that certain phases of metabolism were intimately associated with the responses already recorded.

ABSORPTION AND ASSIMILATION OF NITRATE

The sweet pea plants of these experiments contained an abundance of nitrate in roots, stems, and leaves. The various concentrations of salts employed under the seasonal conditions of these experiments were all such as to furnish the plants with more than an adequate supply of nitrate for vigorous protein synthesis. With the microchemical observations, the total ash determinations (table V) also gave evidence indicating that mineral elements were not lacking in the tissues of the plants of the respective series. In general the nutrient solutions of higher salt concentrations resulted in a definitely higher percentage of nitrate in all organs of the plants concerned (table V), as compared with those supplied with more dilute nutrient solutions. As will be shown in the following pages, however, this was

probably due in part to limited utilization of nitrate in protein synthesis, with consequent accumulation of the unelaborated nitrogenous nutrient. EMMERT and BALL (11) also found that with decrease in moisture content of soils there was increase in the percentage of nitrate found in the tissue of tomato plants.

Whereas the several concentrations of salts of the various nutrient treatments permitted adequate absorption of nitrate and other nutrients, they indirectly influenced very greatly the capacity of the roots and tops to reduce nitrate to nitrite, ammonium, and amino acids. This process of reduction and synthesis has been termed *nitrate assimilation* (20). The relative rate at which this phase of protein synthesis takes place is indicated with considerable accuracy by ECKERSON'S method (7, 20), which "consists of taking an aqueous extract of fresh plant tissue and measuring the amount of nitrite reduced from nitrate by a given sample under specific conditions of time, pH, and temperature. The amount of nitrite formed from nitrate gives a measure of the *reducase* activity of the particular plant or organ sampled." Analyses of plant material (9, 18, 20, 22) have demonstrated that reducase activity closely parallels the synthesis in the plant of amino acids and other forms of organic nitrogen. Reducase activity therefore makes available a convenient index of the relative rate of nitrate assimilation.

The results of such determinations are shown in table IV. The roots of sweet pea were consistently low in ability to assimilate nitrate, as compared with perennial plants such as apple (8, 20, 22), asparagus (26), and narcissus (25); but as in the case of apple (20), it was the young succulent tips which were most active in nitrate reduction, not the older roots which were less succulent and contained much less protoplasm. The roots of the plants of the cultures receiving the more dilute nutrient solutions (one half or one atmosphere) were higher in reducase activity. These results were not surprising, however, as it will be recalled that these roots were slow in maturing; the cortex persisted and many cells retained relatively dense protoplasmic contents for a considerable period. The roots of the cultures supplied with the more concentrated solutions of salts lost their cortex early, and in the central cylinder, cells containing dense protoplasm were few. The prevailing elements of the stele were mechani-

cal fibers and strongly lignified xylem (figs. 3–9). Such tissues are notably lacking in ability to synthesize proteins from nitrate (**20**).

That a similar situation prevailed in the stems is also clear (table IV). The most vigorous reduction of nitrate occurred in the younger portions of the stem where there was present a higher proportion of cells containing protoplasm. Proceeding from the tip to the base of

TABLE IV

REDUCTION OF NITRATE BY EXTRACTS OF DIFFERENT PORTIONS OF SWEET PEA PLANTS (SAMPLES FROM MAIN AXIS; STEM TIP TO ROOT TIP AT POINTS INDICATED)

	Approximate concentration of nutrient solution in atmospheres							
	Dec. 8, 1934				Jan. 4, 1935		Jan. 7, 1935	
	½	1	2	3	½	3	½	3
	Nitrogen (mg.) as nitrite per gram fresh weight							
Distance from stem tip (cm.)								
Stem tip (first 30 cm.)	0.018	0.020	0.013	0.010	0.047	0.019	0.032	0.012
90	0.027	0.031	0.005	0.009	0.036	0.012	0.030	0.010
150	0.015	0.011	0.001	0.002	0.015	0.007	0.007	None
210	0.009	0.008	None*	None*	0.009	Trace	0.006	None
270	0.007*	0.007*	.....	.....	0.001	None*	Trace	None*
280	.....	.....	.....	.....	None*	.....	None*	.....
Old roots (diameter 4 mm.)	Trace	None	None	None	None	None	None	None
Old roots (diameter 2 mm.)	0.001	0.001	None	Trace	0.001	Trace	0.001	Trace
Root tips (1–2 cm. in length)	0.004	0.003	0.001	0.001	0.003	0.001	0.002	0.001

* Base of stem

the stem, there was a marked decrease in rate of assimilation of nitrate in all groups of plants. But this decrease was much more marked in the stems of the cultures supplied with the more concentrated nutrient solutions (two and three atmospheres respectively), and quite logically so, as the anatomical studies have shown (figs. 10–15) that there was much more rapid maturity of cells. This resulted in tissues which were strongly developed mechanically but relatively low in proportion of cells containing abundant protoplasm.

Even the sample termed *stem tip*, which included the distal 30 cm. of linear stem growth, was on any given date much lower in reducase activity in the cultures of high nutrient concentration than in those supplied with more dilute salt solutions (table IV). As already shown, however, even at 1 cm. from the tip the stems of the former cultures were further advanced in maturity and relatively low in proportion of opaque protoplasm; consequently they were low in reducase activity.

The matter of variation in reducase activity on different dates is probably associated directly or indirectly with the seasonal light conditions. In tomato at least (**9**), cloudy weather or long nights (short days) definitely decreased the reducase activity. The reason for this is not known, but it should be recorded that there was much more cloudy weather (and of course the nights were longer) in early December preceding the harvest of plants than later in the month just before the determinations were made in early January.

However, although cells lacking abundant protoplasm are low in nitrate reducing capacity, it seems also to be true that tissues are limited in ability to assimilate nitrate if carbohydrates are extremely deficient (**6, 9, 18, 20**). (Obviously reduction of nitrate cannot occur without oxidation of carbohydrates or their derivatives.) It is perhaps significant that the sample designated as *stem tip* of the series of low nutrient concentration was definitely low in sugars when harvested on December 8, and this is the only case where the distal sample of stem tissue was lower in reducase activity than the portion of older tissue of the stem 150 cm. from the tip (table IV). But the latter sample was much higher in carbohydrate content. The stem tip samples of the cultures supplied with more concentrated nutrient solutions were much higher in sugars and fluctuated relatively little in nitrate reducing ability.

The results discussed in the foregoing paragraphs would not seem to indicate that the higher concentrations of salts employed directly limited the synthesis of proteins from nitrate. Rather, a nutrient medium high in concentration of salts is known to limit the absorption of water (**16**). As already recorded, the plants exhibited in external appearance and anatomy responses which were relatively xerophytic in nature. The plants were strongly developed mechani-

cally but lacking in succulence, and contained comparatively few cells with dense protoplasmic contents, a characteristic which is essential for vigorous assimilation of nitrate.

Chemical composition

Nitrogenous fractions.—It has already been pointed out that the percentage of nitrate in roots, stems, and leaves was directly correlated with the concentrations of this material in the nutrient solution. The fact that there were different amounts of nitrate in the several series of plants was probably of little significance in relation to protein synthesis, as there was present in all cases an adequate amount for this phase of metabolism. Nitrate is not an essential part of the living protoplasm but represents an excess of nutrient material not yet assimilated (**25, 26, 27**).

That the rate of synthesis from nitrate of amide, amino, and protein nitrogen was relatively rapid in the plants supplied with the more dilute nutrient solutions has already been indicated by the reducase determinations (table IV), and is further corroborated by the results of macrochemical analysis (table V).

In all parts of the plants, the percentage of total assimilated or nitrate-free nitrogen tended to be consistently higher in the series supplied with the dilute nutrient solutions (one half and one atmosphere) than in those grown with a greater concentration of salts in the nutrient medium (two and three atmospheres). Also there was obviously a much greater absolute amount of elaborated nitrogenous material in the plants grown in the less concentrated solutions, for they were greater in volume (fig. 1) and total green weight (table II). Further, the percentage of ammonium, amino, and amide nitrogen was notably high in all parts of these plants. But this would seem reasonable, as reducase activity was also much higher in these cultures (table IV). In the assimilation of nitrate and synthesis of proteins, the nitrogenous materials mentioned would be among the first products formed and would tend to be present in greatest amount in plants which were most active in this process.

Associated with the comparatively high percentage of these simpler water soluble forms of organic nitrogen, the more complex protein fraction was relatively low (table V) in the plants grown at the

more dilute nutrient concentrations of one half and one atmosphere. This is, however, a situation commonly found in plants that are succulent and growing vigorously (**5, 12, 17, 25, 26, 27, 28, 32**). Proteinaceous material is presumably high in meristematic tissue, but storage proteins do not seem to accumulate in tissues that are low in dry matter. Although protein constituted a comparatively high proportion of the total organic nitrogen of the plants supplied with the

TABLE V

NITROGENOUS AND CARBOHYDRATE FRACTIONS, ASH, AND DRY MATTER IN VEGETATIVE ORGANS OF SWEET PEA, DECEMBER 9, 1934, EXPRESSED AS PERCENTAGE OF GREEN MATTER

	Approximate concentration of nutrient solution in atmospheres											
	Stems and petioles				Blades				Roots*			
	½	1	2	3	½	1	2	3	½	1	2	3
Total nitrate-free N	0.431	0.411	0.354	0.379	0.647	0.620	0.612	0.600	0.142	0.140	0.130	0.118
Protein N	0.236	0.211	0.265	0.304	0.507	0.500	0.539	0.546	0.070	0.079	0.097	0.094
Nitrate-free soluble N	0.195	0.200	0.089	0.075	0.140	0.120	0.073	0.054	0.072	0.061	0.033	0.024
a-amino N	0.052	0.057	0.017	0.025	0.032	0.040	0.020	0.011	0.031	0.022	0.002	0.002
Amide N	0.040	0.051	0.020	0.019	0.020	0.028	0.015	0.014	0.011	0.017	Trace	Trace
Ammonium N	0.004	0.006	0.001	0.002	Trace	Trace	Trace	Trace	0.002	0.003	Trace	Trace
Nitrate N	0.020	0.032	0.058	0.083	Trace	0.007	0.042	0.036	0.010	0.058	0.123	0.182
Total N	0.451	0.443	0.412	0.462	0.647	0.627	0.654	0.636	0.152	0.198	0.253	0.300
Dry matter	15.00	13.60	18.40	18.00	14.00	13.40	17.00	17.00	5.70	6.60	7.90	8.00
Reducing sugars	0.77	1.30	1.41	1.53	0.30	0.26	0.91	1.00	0.70	0.31	1.36	1.27
Sucrose	Trace	0.17	0.15	0.27	0.12	0.05	0.29	0.39	0.00	0.16	0.39	0.74
Total sugars	0.77	1.47	1.56	1.80	0.42	0.31	1.20	1.39	0.70	0.47	1.75	2.01
Starch and dextrin	0.39	0.49	0.81	0.79	0.55	0.63	1.47	1.53	0.28	0.37	0.42	0.31
Total carbohydrates	1.16	1.96	2.37	2.59	0.97	0.94	2.67	2.92	0.98	0.84	2.17	2.32
Ash	0.15	0.14	0.16	0.17	0.18	0.17	0.21	0.22	.....	.....	.....	.....

* Results expressed as percentage of ash-free green matter.

more concentrated nutrient solutions (two and three atmospheres), it will be shown presently that these plants were relatively high in dry matter, including sugars and starch, and low in percentage of moisture. The later stages of the synthesis of proteins, at least of the storage type, presumably involves chemical dehydration, as in the condensation of amino acids to polypeptides. In plant organs, it has frequently been found that when amino acids and similar compounds are available, the synthesis of storage proteins occurs with decrease in percentage of moisture and increase in concentration of carbohydrates in the plant. It is not a result peculiar to effects of high salt concentration in the nutrient medium, however, for many factors of

nutrition and environment may be employed to give an increase in percentage of dry matter and decrease in moisture (**5, 12, 17, 25, 26, 27, 28, 32, 35**).

It may be noted, also, that, as compared with stems, the blades and roots of sweet pea have a very high proportion of their total elaborated nitrogen in a relatively complex protein form (table V). This is not in any sense peculiar to the cultures of these experiments, however, but seems to be generally true of other plants (**4, 26, 27, 32**).

CARBOHYDRATES.—The results of the carbohydrate analyses are shown in table V. Detailed comments would seem unnecessary. Practically without exception, reducing sugars, sucrose, and starch are much higher in all parts of the plants of the less succulent series which were supplied with the comparatively concentrated nutrient solutions (two and three atmospheres).

The relative rate of carbon dioxide exchange in the several series of cultures is unknown. Such determinations would have added materially to the value of the experiments. Nevertheless in the reduction and assimilation of nitrate, there necessarily occurs oxidation of carbohydrates or their derivatives, resulting in a decrease of those in storage unless supplied by new synthesis. The comparatively rapid reduction of nitrate (table IV) by the plants of the cultures supplied with the dilute nutrient solutions (one half and one atmosphere) undoubtedly contributed to the relatively low concentration of carbohydrates found in the tissues of these plants.

General discussion

These experiments on effects of the salt concentration of the nutrient medium were conducted with sweet pea but it would seem reasonable to suppose that other kinds of plants, although varying in degree of response, would react in a somewhat similar manner. There appear to be frequent demonstrations of this fact in the field and greenhouse. As pointed out in the introduction, the more successful growers of greenhouse plants water very sparingly during the winter, when opportunity for carbohydrate synthesis is limited. By so doing they obviously increase the salt concentration of the soil solution, and thereby limiting the absorption of water, obtain early maturity of tissues and a decreased rate of protein synthesis from

nitrate. Carbohydrates tend to be conserved, therefore, and to accumulate as they are used less vigorously in protein manufacture.

The employment of a high salt concentration is in practical effect that of a continuous but low plane of nitrogen nutrition, and plants exhibit the typical characteristics of such treatment. Clearly when opportunity for carbohydrate synthesis improves, as in the longer brighter days of late winter and early spring, the concentration of salts in the soil solution should be diluted by more copious watering. The more successful growers do just this. The continued employment of a high salt concentration in the long bright days of spring resulted in marked symptoms of nitrogen deficiency in a few of the sweet pea plants of these experiments which were allowed to remain after the investigation was discontinued. Plants of the low concentration cultures during the same period became much less succulent and carbohydrates accumulated. They became very similar in character to the high concentration series of midwinter. It would seem that to obtain ideal vegetative and reproductive growth, the protein nutrition of plants must be considered in relation to opportunity for carbohydrate synthesis. By varying the salt concentration with seasonal light conditions through judicious watering of soil-grown plants in the greenhouse, it seems probable that there may be controlled in considerable degree the rate of protein synthesis and quality of growth of plants.

Soil scientists have correctly emphasized the fact that plants can obtain water over a wide range of soil moisture content from the maximum field capacity to nearly the wilting coefficient (**2, 33**). But, as has been shown in these experiments, a high salt concentration may have marked effect on plant growth and metabolism even though salts are not sufficiently concentrated to result in plasmolysis of the root hairs or wilting of the leaves. In an apple or peach orchard, for example, effects of a low supply of moisture in the soil (high salt concentration) would be very drastic long before the moisture supply became low enough so that the leaves would wilt. In apple (**8, 19, 22**), peach (**3, 23**), and in many of our perennial plants (**9, 25, 26**), probably including certain grasses (**30**), the initial stages of protein synthesis occur almost exclusively in the fine suc-

culent rootlets; but in a solution high in concentration of salts, roots rapidly become woody, optically empty, and lose their capacity for protein synthesis. Nitrate absorption is not limited but the roots lack the capacity to assimilate nitrate, that is, synthesize amino acids and proteins. The effect will therefore be that of "nitrogen deficiency," or more accurately, that of protein deficiency.

Although somewhat aside from the subject of this paper, it may be pertinent to point out that long before the wilting coefficient of the soil is reached, there will be not only an increase in total concentration of salts, but with limited moisture some salts will presumably go out of the soil solution sooner than others. EMMERT and BALL (11) found that dry soil caused plants to accumulate in their tissues high concentrations of nitrate, but a reduction of moisture in the soil was associated with a decrease in percentage of phosphate.

Calcium phosphate might well be one of the first nutrient materials to go out of solution in a soil that was low in moisture content. But under most circumstances (21) effects of calcium deficiency occur early and are more severe than effects of insufficient phosphate (7). In New Jersey, apple orchards have frequently been found which exhibited roots that were high in nitrate but which were definitely deficient in calcium, as shown by external symptoms and analyses. Following an increase in water content of the soil, calcium was apparently again brought into solution; at least newly developed roots exhibited no sign of calcium deficiency and contained an abundance of this element.

Summary

In the fall and early winter of 1934, sweet pea plants were grown under usual commercial conditions of temperature and humidity. All of the cultures received a complete nutrient solution which was continuously percolated through the white quartz sand of the self-draining porcelain culture jars. Each group of plants received an abundant supply of nitrogen as nitrate and the same ratio of nutrient salts. Some of the plants were supplied with relatively dilute nutrient solution at concentrations of one half and one atmosphere respectively, others with comparatively concentrated solution at two and three atmospheres respectively.

1. The plants supplied with the more dilute nutrient solutions grew vigorously and were relatively succulent; the leaf blades were thin, somewhat circular in shape, and dark green. A comparatively high percentage of flower buds abscissed.

2. The cultures supplied with the more concentrated nutrient solutions grew less vigorously and were less succulent; the leaf blades were thick, oval or eliptical in shape, and light green. A comparatively low percentage of flower buds abscissed.

3. The roots and tops of the plants grown in the less concentrated solutions were high in proportion of young active cells containing dense protoplasm. All tissues differentiated and matured slowly. Carbohydrates were relatively low and organic nitrogen high. A large proportion of the elaborated nitrogen was in the form of amide and amino nitrogen. Nitrate was present in abundance.

4. All organs of the cultures supplied with the more concentrated solutions were relatively low in proportion of young cells with dense protoplasmic contents. Tissues differentiated and matured rapidly. There were present strongly developed fibers and other mechanical elements. Carbohydrates were high and organic nitrogen relatively low. A large proportion of the elaborated nitrogen was in the form of complex proteins. Nitrate was present in abundance.

5. With a deficiency of carbohydrates and slow maturation of tissues of the low concentration cultures, there persisted at the junction point of pedicel and peduncle several tiers of cells which failed to mature and remained meristematic in appearance. These tiers of cells were approximately at right angles to the main axis of the pedicel. Ultimately the middle lamellae of one or more tiers became gelatinized, accompanying which the cells separated and the flower bud abscissed. This mode of derivation of the abscission layer is in contrast to the abscission through the non-succulent pedicels of mature fruits, such as the apple, or abscission through the comparatively woody petioles of mature leaves, in which case mature cells apparently become active and thus give rise to the tiers of meristematic cells (**29**) forming the abscission layer.

6. It is a well recognized fact that the amount of water absorbed from a concentrated solution of salts is much less than from a more dilute solution (**16**). As already recorded, the plants of the cultures

supplied with concentrated nutrient were lacking in succulence and otherwise xerophytic in character.

7. Although the percentage of nitrate was fairly high in the plants of all the nutrient series, an effect of a high concentration of nutrient salts was practically that of low nitrogen nutrition, or more correctly, low protein nutrition.

8. Only comparatively young cells containing abundant protoplasm were capable of synthesis of proteins from nitrate.

9. As the plants of the high concentration cultures had relatively few young active cells, they exhibited only limited reduction and assimilation of nitrate and there was accordingly much less oxidation of carbohydrates or their derivatives. This fact would seem to account, at least in part, for the marked accumulation of carbohydrates.

10. Relationships of soil moisture content and salt concentrations in the soil are discussed and it is pointed out that, long before the wilting point is reached, the salt concentration of the soil solution may bring about early maturation of tissues.

11. Mature, optically empty tissues being low or entirely lacking in ability to synthesize proteins from nitrate, there is brought about a condition of protein deficiency incorrectly called nitrogen deficiency, since under the conditions mentioned, nitrate may be present in abundance in the nutrient medium and in the plants. The fact is also emphasized that such effects are especially severe in case of fruit trees, some grasses, and other perennials, because such plants carry on the initial phases of nitrate assimilation and protein synthesis almost exclusively in the fine succulent rootlets. These organs mature and lose ability to manufacture protein very rapidly when exposed to a relatively concentrated solution of salts. The roots, however, can absorb nitrate and other nutrients freely if they are present in the solution of the nutrient medium.

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RATIO OF WATER CONTENT TO DRY WEIGHT IN LEAVES OF THE CREOSOTE BUSH

ERNEST H. RUNYON

(WITH NINE FIGURES)

Introduction

The general characteristics of the remarkably drought resistant creosote bush, *Larrea tridentata* Cav., have recently been discussed and the structure and life history of the leaves portrayed (**23**). Part of the foliage is persistent through long drought. The same leaf may actually have two periods of growth separated by a season of drought. In the work here reported it was found that this drought resistant foliage is characterized by an exceptionally low water content and high saturation deficit. Other foliage on the same branches is unable to withstand a decrease in moisture content much below a level characteristic of many mesophytic woody plants. By a simple method it is shown that leaves with the same moisture content may differ markedly in their access to a water supply. The investigations were made in the vicinity of the Desert Laboratory of the Carnegie Institution of Washington, Tucson, Arizona, during the summers of 1928–31.

Literature

Apparently no special study of the water content of *Larrea* has previously been made. LIVINGSTON and BROWN (**15**), the first to publish data in this country concerning water content changes in leaves, give four measurements for *Larrea* foliage (showing a change not confirmed by the present work). SCHRATZ (**25**), in a study of various aspects of the water physiology of Arizona desert plants, includes 12 moisture determinations on leaves of *Larrea* for the purpose of indicating the influence of time of day and of habitat. ASHBY (**1**) has added three more determinations to this short list. The data presented in these papers indicate that the foliage has a low and fluctuating water content, which may be from about one-half to two times the dry weight.

Prominent in the general literature pertaining to the water balance of plant tissues are the publications of YAPP and MASON (41), WALTER (40), MAXIMOV (18), PISEK and CARTELLIERI (22), and STOCKER (31–35). Earlier literature is reviewed by these authors. While many of the methods employed in these studies are open to certain criticisms, the facts in regard to water content stand out prominently. Each species has a somewhat characteristic water content which changes within limits which are also rather well defined. In some species, especially those of dry habitat, changes are very marked; in others, especially shade mesophytes, the water content remains well poised. In any habitat, however, there are likely to be both types of plants, some physiologically capable and some incapable of withstanding wide variations of water content. Inasmuch as fluctuations are due to a varying balance between water intake and loss, they form a valuable index to the water conditions of the plant.

True water content change, however, is rarely measured. Change of *percentage* water content is another quantity. Most often, percentage water is interpreted simply as absolute water content. This may result in magnification or in masking of the true changes of water content. Thus, for example, differences in actual water content are probably less than differences in some of the percentage data of KRASNOSSELSKY-MAXIMOV (19) and of SCHRATZ (25), because of changes of dry weight in direction opposite to those of water. YAPP and MASON (41) even draw conclusions as to turgor differences from their percentage data. The experimental conditions in some of YAPP and MASON'S experiments were certainly such as to induce marked dry weight changes, exaggerating water content differences. MAXIMOV and KRASNOSSELSKY-MAXIMOV (19) have shown in one case how the rapid disappearance of organic reserves in wilting leaves may prevent their showing a percentage water content below that of turgid controls.

Several investigators, using plants of high moisture content (5 to 10 times the dry weight), have expressed their data as percentage of the *fresh weight*. When this is done, extensive changes in actual water content make comparatively small changes in percentage water content, as may be seen in figure 1. The water percentage of the fresh

weight changes progressively less and less as the absolute water content increases if the dry weight is constant. Water as percentage of the dry weight, on the other hand, is a linear relation of the absolute water content (fig. 1). The masking effect of the fresh weight basis of computation makes it seem possible that the actual water content changes in the plants investigated by KNIGHT (**12**) and by GILBERT and ADAMS (**8**) may have been greater than is indicated by their data.

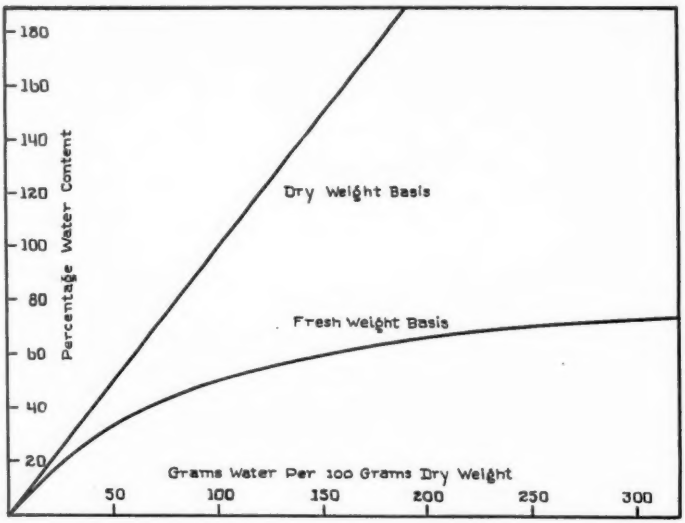

FIG. 1.—Relation between water content and percentage water content. Comparison of dry and fresh weight bases of computation.

The moisture content does change even in shade plants. Percentage water content data are supported by data obtained by other methods, as by volume changes (**2, 36**) and the difference between absorption and transpiration (**29**). DENNY (**7**) has followed diurnal changes in leaves by methods which permit approximate determination of absolute changes. He has demonstrated fluctuations in absolute water content so great as to show that the fresh weight basis of comparison is unsuitable for most of the varieties (about 20) he used. The variations in absolute water content might have been greater had he collected his material before 4 P.M., when the mini-

mum percentage water content frequently occurs. In the woody plants DENNY used, the dry weight did not change appreciably during the night. The numerous correlations which have definitely been established between percentage water content changes and changes in the water conditions are further evidence that true water content changes are responsible for much of the observed percentage changes.

On the other hand, water content is affected not alone by water conditions. Osmotic, hydrational, and wall pressures, which influence the water content of a cell, may be markedly affected by the nature and concentration of the ions present. Water content will therefore vary with the nutrient conditions of the habitat (**14**). Differences in the water conductivity of stems may be expected to influence the water content of leaf cells (**28**). Flowering or tuber formation may possibly influence the amount of foliar moisture (**4, 3, 10**). Finally, light undoubtedly influences the capacity of cells to hold water. Interpretation of the meaning of water content data must therefore be made cautiously. Nevertheless, especially in desert regions where all vegetation is dependent on its water conditions, nutrient and other differences (although of unquestionable influence) are probably of minor importance as compared with water supply in affecting water content.

A small number of water content measurements on any one species can have little significance because of the likelihood of sampling errors and because meaning of the data procured is obscure without bases of comparison, that is, standard water contents associated with some definite conditions or activities of the plant. For interpretation of the existing water content, several investigators have compared it with the value attained when leaf cells are brought to approximate saturation with water (**37, 11, 26, 4, 31–35, 21, 22**). For attainment of saturation, shoots are usually put in moist chambers, their cut stems in water, for 12 to 72 or more hours. VASSILJEV (**39**) objects to this method in that it leads to values which are too high; periods as long as three days in a moist chamber probably do lead to erroneous results. However, the accumulated data give some idea of the degree to which the cell walls normally are distended. Some plants are always far below saturation; others suffer injury if their water content is reduced much below their maximum water holding

capacity. The difference between the percentage water content at saturation and the existing percentage water content has usually been spoken of as the saturation, or water deficit.

OPPENHEIMER (21) recommends determination of sublethal water content, which is the percentage of the saturation water content which may be lost before death of detached leaves or shoots left on a laboratory table perhaps two or three weeks. The likelihood of distinctly pathological effects would seem to restrict the utility of this method. Where measurable, a much more reliable and significant water content is that at permanent wilting (5, 13, 17). Even this minimum water content may be rather indefinite (20), and is not determinable for a plant like *Larrea*, which never wilts and normally shows large changes in moisture content.

Knowledge of the degree of hydration of the chloroplasts, cytoplasm, nucleus, or other portions of the protoplast would be more significant than knowledge of the total "free" water content of tissues. However, as yet we have no method for determining the water content of even the protoplasm alone as distinct from the associated non-living portions. The finest methods are gross in comparison with the intricacy of organization of the living plant.

Methods

Leaves are picked from the bush and put into tared weighing bottles or vials, which are then kept tightly stoppered and as cool as possible. The extreme importance of careful sampling will be evident from the data presented. The inclusion of parts other than leaves (twigs, galls, flowers, or fruits) may introduce considerable error. If weighing cannot be made immediately after picking the leaves, the bottles are inclosed in a humid box. Weighings are made to milligrams. More accurate weighing is unnecessary (except for very small samples) in view of the magnitude of variations inevitable in sampling. After weighing the leaves, the stoppers are removed and the leaves heated in an electric oven for about 24 hours. In most cases leaf samples are completely dry in less than 12 hours, but as one day is a more convenient interval, and since no appreciable change in weight of *Larrea* leaves occurs even with much longer heating, the 1 day period has been adopted. At no time in Tucson

has it been found necessary to use a desiccator; the dry leaf material is not sufficiently hygroscopic to increase significantly in weight even after standing open to the air for 20 minutes. Weighings, however, are always made immediately after the stoppered containers have sufficiently cooled.

To emphasize the possibility of a varying dry weight, data are given for the most part as the ratio of water content to dry weight (wc:dw) instead of in percentage figures. Percentage water content is of course 100 times the value of the ratio. Wc:dw ratios at different times of day, at different seasons, at water saturation, and when transpiration is eliminated are compared. The special methods required for the determination of these ratios are described in their respective sections.

I. The wc:dw ratio under natural conditions

Leaves of different age.—The creosote bush has an abundance of fine branches. One side branch is usually to be found at each node. Under favorable conditions, that is, ample soil water, every branch is densely clothed with tiny leaves, two at a node for seven to ten nodes from the apex. The wc:dw ratio of leaves of different age as found contemporaneously at these different nodes is markedly different. The sampling method used to determine this was to select several (6–20) leafy shoots, and to put all the buds in one weighing bottle and all of the leaves at each successively lower node into separate weighing bottles. In some cases all of the leaves were divided into only two or three groups, each group containing the leaves of two or more nodes from each of the several twigs. All of the data obtained fall into either one or the other of two contrary groups, whose characteristics are summarized in tabulation shown on page 524. Figure 2 shows graphically data selected from the two groups. These two seemingly irreconcilable situations will be discussed later.

Clearly, since so great differences in percentage water content are to be found even at adjacent nodes of *Larrea* stems, uniform successive samples from the same bush can be obtained only by careful selection of leaves of exactly the same age at each picking.

Leaves from different branches of same bush.—The leaf wc:dw ratio of one branch may or may not be closely similar to that

of other branches. Differences as great as 20 per cent may exist between different branches on the same bush. Even when branches are selected for close uniformity, as when two forks ("twins") of a Y-shaped branch are separately sampled, differences as great as 5 per cent are sometimes shown. By very careful selection closer replication is usually obtainable, but in following progressive changes of the wc:dw ratio, differences less than 5 per cent are of doubtful significance.

DATA OF GROUP I	DATA OF GROUP II
—show the wc:dw ratio to be higher at each successively lower node; that is, the ratio increases with age.	—show the wc:dw ratio to be lower at each successively lower node; that is, the ratio decreases with age.
—show a rather consistent uniformly ascending gradient from one node to the next below.	—show not so consistently a uniformly descending gradient from one node to the next below.
—comprise all the determinations made on dates other than Aug. 9–12, 1931, namely, 9 dates between June 15 and Sept. 2, including periods of drought dormancy as well as periods of rapid growth.	—comprise all the determinations made on the four consecutive days Aug. 9–12, 1931, and none other,[1] a period of very rapid growth.
—show wc:dw ratios of 0.63–1.68.	—show wc:dw ratios of 1.20–1.98, the latter being the highest recorded for *Larrea* in the present investigations.
—comprise 63 determinations, involved in 18 tests, made on 12 bushes.	—comprise 60 determinations, involved in 14 tests, made on 7 bushes.

[1] In two of the three tests at the University of Cincinnati, greenhouse-grown *Larrea* showed the maximum wc:dw ratio in the buds. The water content of the buds and youngest leaves was more than twice the dry weight.

VARIATIONS WITH SEASON AND HABITAT.—The purpose of these investigations was to determine the nature of the existing variations of the wc:dw ratio. Except in a general way, no attempt was made to correlate these variations with environmental factors.

Adjacent bushes in an apparently uniform habitat frequently deviate conspicuously in water content, indicating probable local differences in water conditions. Thus for example, within an area

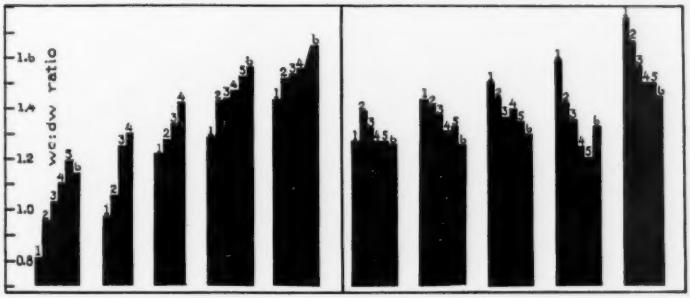

FIG. 2.—The wc:dw ratio of leaves at different nodes. Each diagram represents the relative wc:dw ratio of leaves at successive nodes (1, 2, 3, 4, 5, 6) from apex. The five diagrams at the left represent the usual situation, the oldest leaves having the highest wc:dw ratio; the five diagrams at the right show the reverse situation, which occurred during a period of rapid growth.

of about an acre on Tumamoc Hill six individuals were found to have wc:dw ratios of from 0.71 to 1.13; in another locality six bushes ranged in wc:dw from 0.77 to 0.97. In these tests leaf samples were taken from all parts of the selected bushes, as nearly simultaneously as possible, sometimes with the aid of an assistant. The differences shown are for the most part unpredictable from external appearances, although brighter green foliage frequently has higher percentage water content.

The wc:dw ratios for leaves of bushes in habitats which are obviously different are correspondingly different. In July, 1931, on Tumamoc Hill there had been a number of rains and the ratio was greater than 1.0 (1.10–1.33). At the same time, 8 miles to the south (Ajo Pass, Tucson Mountains) where no considerable rain had fallen, the ratio was little more than half this amount (0.54–0.67). Similar variations occur in the same bush before and after the summer rains.

Determinations made in late June and early July, 1928, on bushes in the vicinity of the Desert Laboratory, showed their average ratio to be 0.54. Following the midsummer rains, the average was 1.35. These data represent 77 tests on 18 bushes. In table I are other specimen ratios of individual bushes before and after periods of rain or watering. Between one growing season and the next, weeks or even months of excessively dry hot weather may intervene, while the percentage water content of all leaves retained on the twigs drops

TABLE I

SEASONAL VARIATION OF WC:DW RATIO

Bush	Before rain period		Precipitation (cm.)	After rain period		Percentage increase
	Date	wc:dw		wc:dw	Date	
L.....	7/26/28	0.59	8.7	1.15	8/15/28	49
8.....	7/31/28	0.59	4.9	1.12	8/ 9/28	47
Bj.....	7/15/31	0.60	10.4	1.30	8/27/31	54
P.....	7/26/28	0.71	6.6	1.30	8/ 9/28	45
b*.....	7/31/30	0.74	*	1.25	9/26/30	41
y*.....	7/27/30	0.89	*	1.95	8/14/30	54
a*.....	7/ 4/30	1.04	*	1.56	7/31/30	33

* Artificially watered.

sharply. If the dry period is long and severe, the water content may fall to less than one-half the dry weight.

From the data on nodal and seasonal differences, we may conclude that a leaf ordinarily undergoes an increase in percentage water content as it comes to maturity, and then a decrease due to seasonal drought.

DIURNAL VARIATIONS.—Several hundred ratio measurements were made during the summers of 1928, 1930, and 1931 in order to obtain a complete picture of the changes that take place from hour to hour of the day and night. Since the simultaneously existing differences from node to node or from branch to branch are often greater than in the same leaves from hour to hour, sampling must be done with the greatest care. One satisfactory procedure is to select several shoots which by previous test have proved to have nearly identical leaf wc:dw ratios, and which have the same exposure, density of

foliage, etc.; to use then the leaves of one shoot for each time of day a sample is desired.

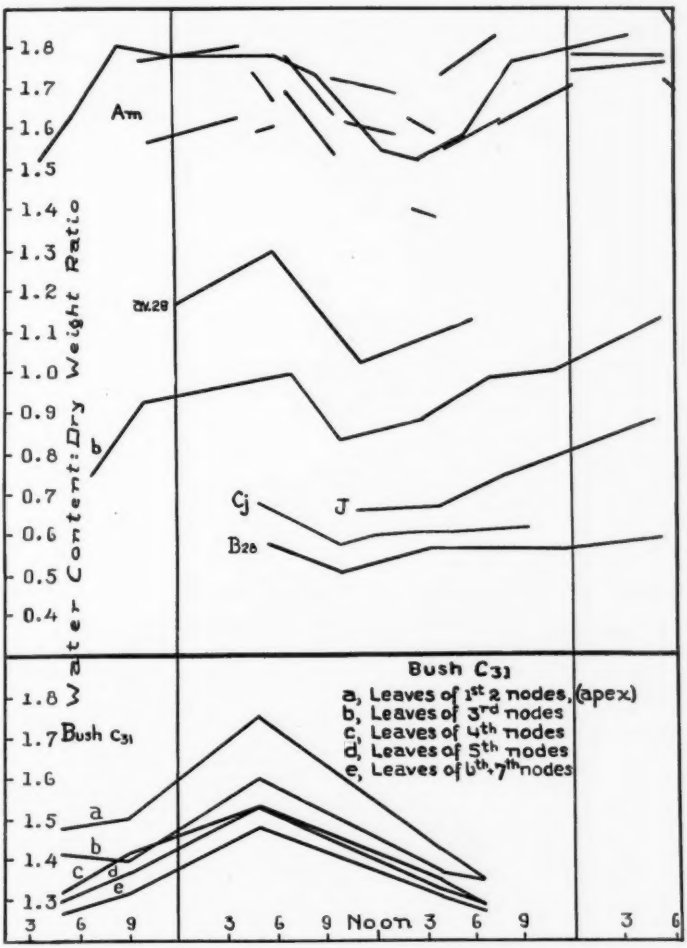

FIG. 3.—Diurnal variation of wc:dw ratio of leaves of different bushes

Graphs representative of the most carefully executed experiments are shown in figure 3. Many other graphs obtained follow the same

curves; a few are of different form, or show erratic fluctuations. The latter results are probably due partly to faulty sampling, and partly to obscure circumstances which derange the normal changes. The curves for bush Am in figure 3 consist of (1) a continuous line curve each point of which represents the water-dry weight ratio of the leaves of a single twig, all of the twigs having been chosen for strict uniformity; and (2) short lines, each of which joins the wc:dw ratio of half of the leaves of a selected shoot at one time and the wc:dw ratio of the remaining leaves 2–6 hours later. The short lines rarely coincide in position with that of the continuous line curve, indicating again that the level of percentage water content may be markedly different in various parts of a bush. It is significant that the same general direction of change appears consistently in all the curves for bush Am. The changing ratio in leaves *at different nodes* is shown in the curves for bush C31. Here all the shoots used had been shown to be uniform in wc:dw ratio. It will be seen that these readings were taken when all bushes tested had a lower ratio in the lower leaves. The other curves of figure 3 represent the average condition of leaves of all ages, and taken from all parts of the bushes indicated. It is clear that inasmuch as these do not show the variations at different nodes and branches, they represent an incomplete picture. A composite of curves must be given to express diurnal variation adequately.

The wc:dw ratio is greatest at daybreak. A sharp decline in the ratio begins at sunrise, leading to a minimum value which may occur at any time between 10 A.M. and 7 P.M. If the minimum occurs early in the day, there is a gradual increase during the afternoon or a low fluctuating ratio until sunset when there starts a steady increase. The difference between the maximum and minimum values for any 24 hour period is from about 0.03 to 0.60, or 10 to 35 per cent of the maximum value.

SEPARATE RÔLES OF WATER CONTENT AND OF DRY WEIGHT IN CHANGES OF THE WC:DW RATIO.—It is to be expected that dry weight changes, if any, are in a direction opposite to those of water content. This follows from a consideration of the usual periodicity of transpiration and photosynthesis. Some evidence in regard to the occurrence of dry weight changes in *Larrea* is afforded by I_2KI-starch

tests made on leaves picked at different times of day. It is found that leaves of irrigated bushes contain conspicuously more starch at 5 P.M. than at 5 A.M. Leaves of bushes not irrigated contain very little starch, however, and exhibit a very inconspicuous difference in the starch content at 5 P.M. and 5 A.M. A change in starch content does not necessarily mean a change in dry weight. But there is a suggestion here that leaf dry weight changes may be more pronounced in bushes amply supplied with water.

A number of attempts were made in the field to determine how much change takes place in the absolute dry weight and water content from hour to hour. In order to do this, one leaf of the pair at each of several nodes was picked at one time, and the other leaf at the same nodes, closely matched as to size and exposure, picked at the second time. Obviously success in such work, especially with such small leaves, requires the utmost care to insure exact replication. Uncontrollable environmental conditions and limited time prevented satisfactory results in Tucson, in lieu of which similar experiments were undertaken at the University of Cincinnati under more favorable, but artificial, conditions, and with very limited material. The plants used were in pots placed out of doors and fully exposed to the sun and wind. The results are given in tables II and III.

As seen in the tables, the two leaves at the same node are designated *a* and *b*. In further experiments, the half-leaf method was used, the two pinnae of the same leaf being separately tested and designated *a* and *b* respectively. Table II shows how closely similar *a* and *b* are when sampled simultaneously. Table III gives the values at about the time the ratio is at maximum and at minimum. Although the data in the tables are meager, some facts seem clear:

1. Except in experiment 1 (in which only three leaves were included in a sample) and in experiment 3, the two leaves at the same node or the two halves of the same leaf as sampled simultaneously differ from each other less than 3 per cent.

2. In experiment 3, the higher difference (4.4 per cent) in the dry weight and milligrams of water does not appear in the percentage water content, making it seem very probable that equivalent areas were not included in *a* and *b;* that is, that there was an error in sampling.

3. The differences shown in table III are greater than those in table II. They are, moreover, in the expected directions. The data are therefore sufficient to demonstrate a diurnal change in dry weight, as well as in water under the conditions of the experiments.

4. Except in experiment 6, the change in water content is greater than the change in dry weight.

TABLE II

COMPARISON OF THE TWO LEAVES (A AND B) AT SAME NODE AND OF THE TWO HALVES (A AND B) OF SAME LEAF (UNIVERSITY OF CINCINNATI)

EXPERIMENT NO. AND METHOD	DRY WEIGHT			WATER CONTENT			WATER CONTENT (% DRY WEIGHT)		
	A (MG.)	B (MG.)	PERCENTAGE DIFFERENCE*	A (MG.)	B (MG.)	PERCENTAGE DIFFERENCE*	A (%)	B (%)	PERCENTAGE DIFFERENCE*
Opposite leaves (a and b):									
1. Leaves of 3 nodes	5.8	5.6	3.50	12.1	12.7	4.84	209	227	8.25
2. Leaves of 6 nodes	8.8	8.9	1.13	19.1	19.0	0.52	217	214	1.39
Average per leaf (9 nodes)	1.622	1.611	0.68	3.467	3.522	1.56	214	218	1.85
Half leaves (a and b):									
3. 10 half leaves	11.5	11.0	4.44	20.8	19.9	4.41	181	181	0
4. 10 half leaves	13.1	13.2	0.76	26.3	26.8	1.88	201	203	0.99
5. 15 half leaves	17.3	16.8	2.93	32.1	31.7	1.88	187	189	1.06

* In percentage of $\frac{a+b}{2}$.

5. Water content and dry weight changes are in opposite directions as predicted. The wc:dw ratio therefore changes more than does the actual water content.

Further information regarding the diurnal changes in *Larrea* leaves is afforded by study of the osmotic concentration of the cell sap.

DIURNAL CHANGE OF OSMOTIC CONCENTRATION AND WC:DW RATIO.—Dr. T. D. MALLERY and the writer conducted an experiment to determine the nature of the diurnal fluctuation of osmotic and wc:dw ratio values (**16**). Two experimental bushes, Am and Bm, were selected as representing contrasting foliage characteristics.

Recent rains had provided enough soil water to permit growth, but Bm appeared less favorably situated than Am. The leaf size and rate of growth were evidently greater for bush Am. (WALTER's (40) figure 26, p. 65, shows Am). At intervals of 2–4 hours for 50 hours, leaf-plus-twig samples were taken from all over the bushes for osmotic and water content data. Simultaneously, leaf samples from care-

TABLE III

DIURNAL CHANGE OF DRY WEIGHT, WATER CONTENT, AND PERCENTAGE WATER CONTENT (UNIVERSITY OF CINCINNATI)

EXPERIMENT NO., METHOD, AND TIME INTERVAL	DRY WEIGHT (MG.)			WATER CONTENT (MG.)			WATER (% OF DRY WEIGHT)			WATER (% OF FRESH WEIGHT)		
	A	B	CHANGE*	A	B	CHANGE*	A	B	CHANGE*	A	B	CHANGE*
Opposite leaf method: 6. 9 pairs of leaves 5:00 P.M.(=a) 4:30 A.M. (=b)	23.6	21.7	−8.4	37.9	39.1	+ 3.1	160.0	180.0	+11.8	59.5	64.8	+8.5
Half leaf method: 7. 10 leaves 5:00 P.M.(=a) 4:30 A.M. (=b)	9.5	9.0	−5.4	14.0	16.6	+16.3	147.0	184.0	+22.2	61.6	64.3	+4.3
8. 10 leaves 5:00 P.M.(=a) 4:30 P.M. (=b)	11.9	11.4	−4.3	18.8	21.3	+12.2	158.	187.0	+16.4	61.2	65.1	+6.2
Average (6+7+8) 5 P.M.–4:30 A.M.	15.0	14.0	−6.5	23.6	25.7	+ 8.2	155.0	184.0	+17.0	60.8	64.7	+6.2
9. 13 leaves 6:00 A.M.(=a) 3:30 P.M. (=b)	16.3	17.0	+4.2	31.6	27.4	−14.2	194.0	161.0	−19.0	66.0	61.7	−6.7

* In percentage of $\frac{a+b}{2}$.

fully selected individual branches were taken for wc:dw ratio determination. Some of the results are plotted in figure 4 (*cf.* MALLERY's fig. 5).

Evidence in regard to diurnal change of dry weight lies in the constancy of the product: osmotic value times wc:dw ratio (22). The product may be designated thus:

$$\frac{kc}{Ws}\left(\frac{Ws+Wr}{c+d}\right),$$

where c = number of millimols of osmotic species, (c+d) = grams dry weight, Ws = grams of solvent water, (Ws+Wr) = grams total water content, and k = a constant. If the solvent water and the total water content are almost equal in the leaf tissue, that is, if Ws and (Ws+Wr) in the product balance each other, then it follows that the product is not much affected by changing water content. On the other hand, total dry weight is undoubtedly considerably greater

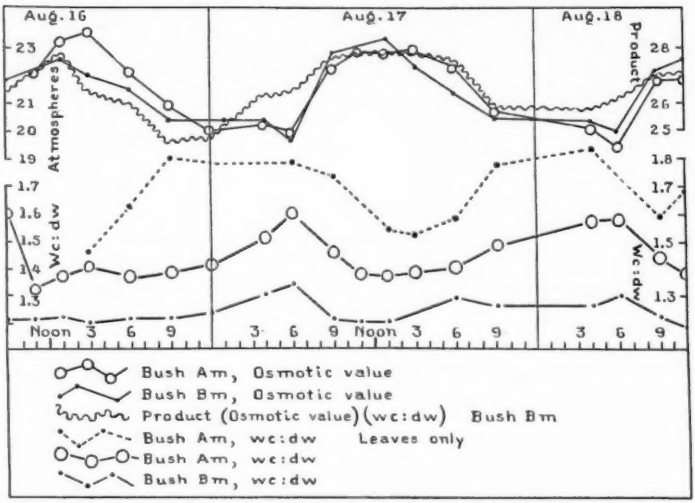

FIG. 4.—Diurnal changes in foliage (leaves plus green twigs) of two bushes, Am and Bm.

than the osmotic portion of it, so that if the product varies considerably, the dry weight is changing (as well as, probably, the water content).

In the present experiment the product varied quite as much as did the factors of the product (fig. 4). According to the foregoing, this means that in these bushes diurnal changes of the dry weight were occurring. Since the validity of the assumptions involved in this interpretation is open to some question, the data must be considered not as proof but merely as evidence additional to that presented in the previous section, pointing to the same conclusion, that diurnal dry weight fluctuations do occur in *Larrea* bushes well supplied with water.

The course of the diurnal changes need not be discussed further than (1) to call attention to the inverse direction of change of osmotic and ratio values, (2) to note that the interpretation (as by WALTER 40) of such a relationship to mean that water content changes are the cause of the osmotic changes is unjustified in view of the probable dry weight fluctuations, and (3) to note that the changes shown are in the directions which would be expected in view of the periodicity of photosynthesis and transpiration.

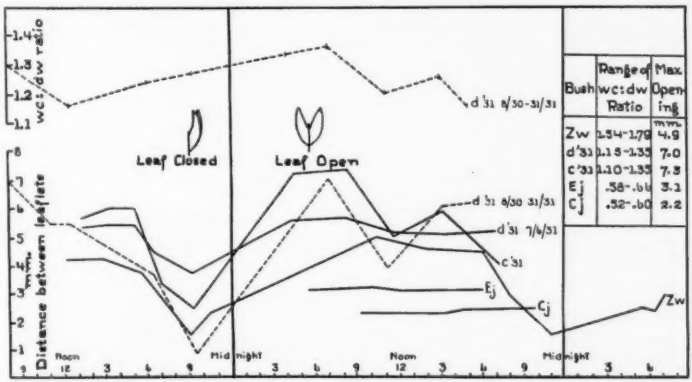

FIG. 5.—Diurnal movement of *Larrea* leaflets and representative curve of the march of percentage water content. No relation exists between time of leaf opening or closing and level of percentage water content.

LEAFLET MOVEMENT; RELATION TO DIURNAL CHANGE IN WC:DW RATIO.—*Larrea* exhibits nyctinastic leaflet movements. In the hope of finding a correlation between the march of diurnal water content variations and the movements, as reported by TRELEASE (38) for palm leaves, exact measurements of the positions of *Larrea* leaflets were made at various times of day and night, and concurrently, wc:dw ratio determinations on similar leaflets. No correspondence appeared, however. The time of maximum opening (7–11 A.M.) occurs two or more hours after the time the water content is at its maximum (sunrise); and the time of minimum opening (7–11 P.M.) occurs many hours after the time of day water content is at a minimum (fig. 5). Evidently the changes in water content responsible for the movements are localized in the motor tissue, and are not general throughout the leaf.

General discussion.—*Larrea* foliage exhibits some striking characteristics. Most outstanding are the changes that may take place in the life cycle of a single leaf, and the lowness of the water content. Analysis of the wc:dw data here reported clarifies and amplifies the concepts previously presented (**23**) showing *Larrea* to have a peculiar admixture of xeric and mesic characteristics.

During the growing season the leaves have a water content level almost identical with that of leaves of such genera as *Quercus*, *Castanea*, *Hamamelis*, and *Fagus* (**27, 6**): between one and two times the dry weight. Indeed most of the foliage at this season in water content as well as in structure gives no evidence of the xeric nature of *Larrea*. Drought resistance is characteristic of only a small proportion of the leaves.

It is possible to construct diagrams showing roughly the wc:dw ratios for representative pairs of leaves that develop successively on a twig, and the change in the ratios from one season to the next during the summer (fig. 6). This is done by correlation of the nodal and seasonal wc:dw ratio data with the life duration and growth studies (**23**), in which it was established that seasonal changes represent not merely a substitution of new leaves for old ones, but an actual change in the same leaves from one season to the next. For convenience, and according to their time of development, three types of leaves, *a*, *b*, and *c*, may be distinguished.

The *a* type of leaf begins its development early in a growing season and is thus subject during its whole developmental period to relatively mesic conditions. Loss of much water is fatal to leaves of the *a* type, and they therefore fall early in the dry period or early in the next growing season. The data on nodal variation show that younger leaves regularly have a lower water content than older ones. The leaves designated *b* and *c* are therefore represented in figure 6 with narrower bands than the *a* leaves, which have previously expanded from the bud. Examples of the *b* type of leaves occur infrequently. They do not attain full size before the dry season checks further growth. They survive the drought and show increased water content but no further growth during the second growing season. It is the *c* leaves which have two periods of growth. These leaves begin their development during a period of increasing drought, last through the dry season with the lowest water content of any of the

foliage, and on the return of rain, not only increase their water content but resume growth. They are shed in the subsequent dry period. The three leaf types listed in the order *a*, *b*, and *c* are arranged according to increasing life duration, increasing drought resistance, and decreasing water content. Of all the leaves, the *a*1 subtype (fig. 6) is most abundant. They have a comparatively high wc:dw ratio

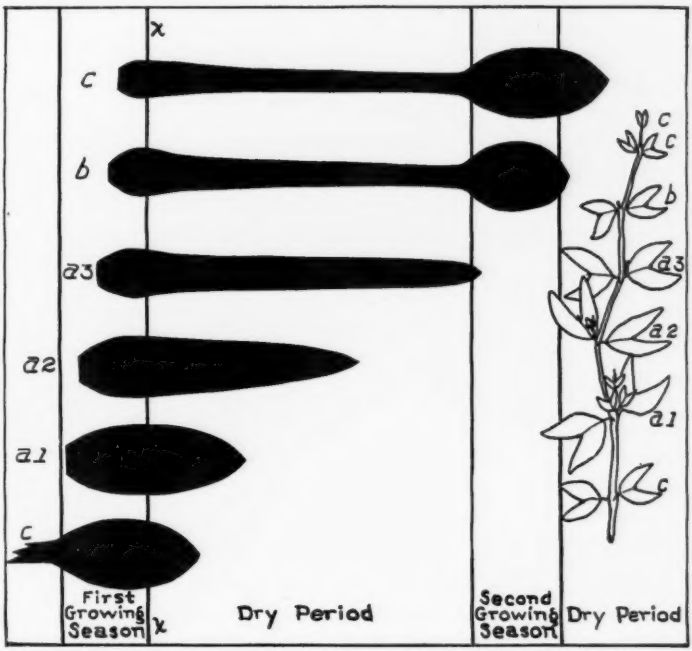

FIG. 6.—Schematic diagram representing life span and percentage water content of *Larrea* leaves. Relative water content indicated by thickness of bands: where the bands become narrower the water content is decreasing, and vice versa. The twig shows the relative positions of different leaf types at end of a growing season (at time indicated by x on left side of figure).

throughout their brief lives and are not drought resistant. The drought resistant *c* leaves look exactly the same except for their small size.[2]

[2] Figure 6 simplifies the true picture of percentage water content changes in the life of a leaf. Diurnal variations are not shown; the summer dry seasons are often broken by

It is to the *b* and *c* leaves that *Larrea* owes its reputation of being the most extreme xerophyte of the southern deserts of North America. The *c* leaves during a severe drought have a water content which is lower (except for air-dry plants or plant parts) than any other found reported in the literature, less than 50 per cent.[3]

Any *a* leaves (*a*2, *a*3) which are retained during a portion of the drought have also a very low water content, not very much more than the *c* leaves adjacent to them at the stem apex. Tremendously different in physiological capacity, these two leaf types are nevertheless quite similar in appearance, structure, and even in water content. In this respect *Larrea* differs from one of its companion species, *Encelia farinosa* (**26**).

A study of the degree of vacuolization and the chemical characteristics of the protoplasm of the *c* leaves as compared with that of the similar appearing but non-drought resisting *a* leaves might lead to a better understanding of the difference between these contrasting leaf types. Early development during a time of water shortage seems to engender drought enduring capacity, while attainment of maximum size and water content entails the loss of this capacity.

The degree of dormancy of the *c* leaves during the drought is of interest. With a water content about one-half their dry weight, metabolic activity must be definitely greater than that of air-dry leaves or seeds whose water content may be no more than one-tenth their dry weight. The appropriateness of the term "anabiosis" as applied to the condition of *Larrea* leaves (**18**) is thus questionable. The data on diurnal variation demonstrate that even in this state of minimum water content, *Larrea* leaves exhibit some diurnal varia-

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short periods of rain sufficient to result in water content increases, but not in growth. The time of occurrence and lengths of the rainy and dry seasons are different each summer. The general situation is as shown, however. June and July are usually the driest times in summer, and after early August rains another season of high temperatures and very low humidity is likely to occur. Even locally, however, there are apt to be conspicuous differences in the time and amount of precipitation.

[3] Water contents lower than half the dry weight are reported by THODAY (**37**) for some microphyllous South African ericads (*Passerina* spp.) but these data are for whole shoots, leaves, and stems together. Twigs of *Larrea* always have a lower water content than the leaves (fig. 4); and the older the stem, the lower the water content (unpublished data).

tion of the wc:dw ratio. No matter how much of this variation is due to dry weight change, fluctuation of the ratio implies activity. Experiments with moist chambers in the field (*q. v.*) indicate that the transpiration rate must be low corresponding to a very slow water intake. The significant fact, however, is not that water content and the rate of transpiration are low, but that some activity, some transpiration does occur; the leaves do not become air-dry.

Nodal differences may be due to age, position, and history. In some cases, as when comparing *a*1 and *a*2 leaves, the difference in age from node to node is slight, a matter of a few days only. In other cases newly developed leaves (*a*) are contrasted with leaves of a former growth period (*c*) which may therefore be months older. In any case, the difference in leaf wc:dw ratio from node to node is of much smaller magnitude than in the same leaf from season to season. In other words, changes due to external conditions are greater than those due to internal development. That the water content increases with advancing age indicates that the amount of water held by a leaf increases faster than does the weight of dry substance. When the cells have attained maximum size, further increase in the ratio must be due principally to dry weight loss. According to WALTER (**40**), the osmotic concentration also increases with age. This leads to the interesting deduction that the dry weight loss must be of the organic constituents, and more than enough to balance the increase in osmotic substances. A similar gradient in the percentage water content from younger to older leaves has been reported (**41**) for various plants, especially herbs. The second maximum (which according to YAPP and MASON occurs in buds, the minimum moisture content being in partially developed leaves) has been found in *Larrea* in only one test.

The reversed gradient of water content (decreasing with age) found during the four days of August 9–12, 1931, was exceedingly perplexing. The inconsistency with former results stimulated great care and effort to establish the true nature of the situation. A clue is afforded by some experiments, to be described later, with shoots in moist chambers. It was found that the youngest leaves have much the greatest water holding capacity, owing probably to their greater wall plasticity. During August 9–12 it may be supposed

that the plants had more than ample water to provide for transpiration. Their water content may thus have been conditioned principally by the extensibility of their cell walls. A descending curve of water content percentage from young to older leaves, even with constantly moist conditions, is reported to be the normal situation in many genera (**27, 17, 22**), but in *Larrea* is probably an unusual occurrence, being evidence of a superabundant water supply.

The characteristics of the diurnal changes are very evidently related to water conditions. The early day decrease and nocturnal increase are undoubtedly related to the changing rate of transpiration. On rare cloudy or rainy days the ratio does not sink so low. The diurnal minimum occurs late in the day more frequently in bushes of high water content, and earlier in the day in dry bushes. The interpretation of this situation may be that in drier bushes increasing water deficit *earlier in the day* reaches a point at which the colloids of the leaf tissues are in approximate equilibrium with their double environment, the taut tracheal sap and the desert atmosphere. Only a certain proportion of the total water content is seemingly subject to evaporation; this proportion is a larger quantity in bushes of higher water content; hence the turning point in the diurnal curve is reached later in the day in these bushes. Further, the magnitude of the diurnal change is related to the level of the water content. When the latter is only about 50 per cent of the dry weight and there has been no rain for months, the diurnal fluctuations are relatively slight, about 10 per cent of the early morning value; whereas when the water content is somewhat greater than the dry weight, the proportionate changes in the ratio may be more than 30 per cent, although more commonly about 20 per cent. Leaves of still higher water content show less proportionate diurnal change than leaves of medium water content; in the former, the water supply is evidently more nearly adequate to maintain an even water balance.

Probable changes of dry weight make the data relative to diurnal variations of the wc:dw ratio of only qualitative significance as regards water content. Diurnal changes of dry weight in semidormant drought-exposed leaves are probably less than for leaves of well watered plants in the relatively cool and humid environment of Cincinnati, where a small change in the dry weight of *Larrea* was demon-

strated. From three lines of evidence, (1) the Cincinnati experiments, (2) the correlation of water conditions with changes in the ratio, and (3) the lack of diurnal change of starch content in leaves of low ratio, the assumption seems justified that in the desert, especially in drier seasons, changes of the ratio are due much more to water content changes than to dry weight changes. During seasons of rain, however, when there is sufficient soil water to permit growth, dry weight as well as moisture undoubtedly undergoes diurnal fluctuations in direction opposite to that of moisture fluctuations, so that the ratio changes more than does the actual water content.

Diurnal variation of foliar moisture in *Larrea* is of about the same magnitude as for many other plants which come under WALTER's classification (**40**) of *euryhydric* species. These are unstable in percentage water content and sap concentration. Here belong such herbs as sunflower and potato (**19**), steppe plants such as *Plantago media* (**11**), and many desert plants (**39, 25, 31, 19, 15**). Contrasted with the euryhydric plants are those which are called by WALTER *stenohydric*, that is, having a very stable water balance. In the desert as in many other habitats euryhydric and stenohydric plants grow side by side.

A striking discrepancy appears (fig. 4) between the osmotic and the ratio values for the two experimental bushes Am and Bm. The level of the wc:dw ratio and the magnitude of its variation are greater for Am than for Bm; but the osmotic concentration of the sap is essentially the same in the two bushes, and follows conspicuously the same course. The difference between the two with respect to leaf size, vigor, and general appearance is paralleled by a difference in the wc:dw ratio, but not obviously in the osmotic concentration of the sap. These results emphasize the inadequacy of osmotic measurements alone in a study of water relations.[4]

It is impossible to assign definitely the reason for this discrepancy between osmotic and ratio values without supplementary data. The

[4] MALLERY (**16**) points out that the osmotic maximum occurs later in the day in bush Am (3 P.M.) than in Bm (1 P.M.), and that the variation in osmotic value is greater in Am. But these differences are certainly less striking than the close similarity of the osmotic curves for the two bushes, whereas the curves for the wc:dw ratio are not only of obviously different amplitude for the two bushes, but are on two distinct levels.

difference between the two bushes may be in water content, dry weight, or in both of these. Both chemical and anatomical investigations are required to give an adequate knowledge of water relations.

PISEK and CARTELLIERI (**22**) and OPPENHEIMER (**21**) have amplified their osmotic data with determinations of the percentage water content, and find in general a good correlation in the changes of the two quantities. In both these investigations, however, occasional discrepancies appear. For instance OPPENHEIMER reports that the stenohydric *Olea* undergoes more water loss than is indicated by the small rise in osmotic concentration; *Ceratonia* likewise may exhibit wilting phenomena but no concurrent increase in sap concentration. Chemical analyses of sap (**30, 9**) have shown that seasonal variations of osmotic value are due in some species (as *Ilex aquifolium, Hedera helix, Pinus sylvestris*) to sugar, in other species to both sugar and water (*Taxus baccata*), while in still others the variations may be due solely to water (*Buxus sempervirens*).

II. THE WC:DW RATIO UNDER EXPERIMENTAL CONDITIONS

THE WC:DW RATIO AT APPROXIMATE SATURATION.—To provide additional basis for comparison of naturally existing water content ratios, determinations were made of the values attained when shoots were inclosed in moist chambers. Some idea of the relation of the existing water content to that attained when the cells are distended to approximately their maximum size is afforded by use of the following method. Two branches are chosen, the moisture content of whose leaves by previous test has been found to be essentially the same. The leaves of one branch are picked for determination of the existing wc:dw ratio. The other branch is cut from the bush while the stem is held under water. The cut end of the severed shoot is kept under water while the whole is inclosed in a saturated atmosphere at a constant temperature for one or 24 hours, when the wc:dw ratio is determined. The data are not all strictly comparable because at times in the field it is necessary to deviate from the procedure just outlined. Two sources of error are thereby encountered:

1. The leaves picked for measurement of the original wc:dw ratio may not be strictly comparable with the leaves brought to saturation. A difference in the proportion of young to older leaves is espe-

cially to be avoided, because young leaves have a much higher water holding capacity.

2. The water content after 24 hours probably does not approach saturation to the same degree in all cases. The moist chambers used provide only an approximately saturated atmosphere. Some temperature changes are often unavoidable. Condensed moisture is sometimes visible as a film on glass or a mirror placed in the chamber. Moisture is never clearly discernible on the leaves, but for evaporation of any film that may have been present a period of exposure to dry air was often provided. An error in judgment as to the proper length of time for this would make some difference in the 24 hour ratio. The increases in the ratio are so great, however, that the errors involved are relatively insignificant.

Data relative to the saturation percentage water content, the amount of increase necessary to attain the 24 hour water content, and the rate of increase are presented in table IV and in figures 7 and 8.

Saturation water contents are found to be high above the naturally existing water contents, the latter being from less than one-third to only about three-fourths of the saturation values. The 24 hour ratio varies from bush to bush, with time of day, and with age of the leaf. Maximum values for the saturation ratio were obtained for bushes (Tb5 and Cinti, table IV) which had a predominance of young, actively enlarging leaves. While in general higher original ratios increase to higher saturation values, often it happens that of two bushes the one with the lower water content is closer to saturation. The existing ratio is no indicator of the saturation ratio.

The 24 hour *increase* is greater in leaves of low water content. Expressed in terms of the original ratio, the increase is very much greater in drier bushes (fig. 7). Thus, according to the data in table IV, wc:dw ratios 0.40–0.70 increase 2-300 per cent, while ratios originally over 1.27 increase less than 100 per cent in 24 hours. In all cases, however, a very considerable increase has been found. The increases represent from 23 to 77 per cent of the 24 hour ratio.

The *rate* with which water enters the stems of cut shoots is of special interest. Figure 8 shows that after the first few hours in the moist chamber the saturation value has already been quite closely

approached, and that thereafter the ratio increases very slowly to the 24 hour value. After 24 hours the ratio continues the very gradual increase, although at 48 hours in some cases a lower value than at 24 hours has been found. Derangement of the normal metabolism of

TABLE IV

CHANGE IN THE LEAF WC:DW RATIO OF CUT SHOOTS IN A MOIST CHAMBER

BUSH	DATE (1931)	WC:DW RATIO			24 HR. INCREASE IN % OF ORIGINAL W.C.	ORIGINAL W.C. IN % OF 24 HR. W.C.
		ORIGINAL	AFTER 1 HR.	AFTER 24 HR.		
Cj	July 23	0.47	.....	1.76	274	26.7
Cj	July 15	0.58	.....	1.73	196	33.3
Cj	July 18	0.59	1.08	1.72	193	34.1
mn	June 19	0.48	1.42	2.08	334	23.0
Bj	July 9	0.57	1.27	2.03	257	28.0
Bj	July 9	0.61	1.32	2.12	248	28.7
Bj	July 9	0.55	1.27	2.03	270	27.0
Bj	July 11	0.62	1.30	2.15	246	28.9
Ej	July 21	0.57	.....	2.26	296	25.1
J	July 18	0.67	1.35	1.98	195	33.8
J	.....	0.68	1.44	1.97	190	34.4
U_{31}	June 27	0.73	1.45	1.93	164	37.8
b	June 18	0.97	1.82	2.18	125	44.8
m_2	July 4	1.01	1.49	2.18	116	46.4
m_1	July 4	1.02	1.46	2.07	103	49.4
e	July 4	1.27	1.91	2.22	75	57.2
e	July 4	1.33	1.69	2.23	68	59.6
C	July 10	1.33	1.73	2.30	65	57.8
E	Sept. 4	1.57	.....	2.76	76	56.9
E	Sept. 4	1.27	.....	2.50	97	50.8
E	Sept. 4	1.17	.....	2.26	93	51.7
E	Sept. 4	1.32	.....	2.38	83	55.5
Am	Aug. 16	1.46	.....	2.52	73	57.9
Am	Aug. 17	1.78	.....	2.40	35	74.1
Am	Aug. 17	1.52	.....	2.54	67	60.0
Am	Aug. 17	1.77	.....	2.29	29	72.3
Am	Aug. 18	1.83	.....	2.48	36	73.8
Am	Aug. 18	1.59	.....	2.46	55	64.6
Tb_5	Aug. 12	1.78	.....	3.02	70	59.0
Cinti	July, 1933	1.94	.....	3.02	56	64.3

these cut shoots in a dark saturated atmosphere for periods longer than 24 hours makes doubtful the significance of 48 hour wc:dw values. The 1 hour percentage increase is roughly proportional to the 24 hour increase (fig. 7). Forty to 70 per cent of the total increase occurs during the first hour, and according to two tests (the

only ones made), 95 per cent of the 1 hour increase takes place within the first 15 minutes. Rapid increase of such magnitude in the wc:dw

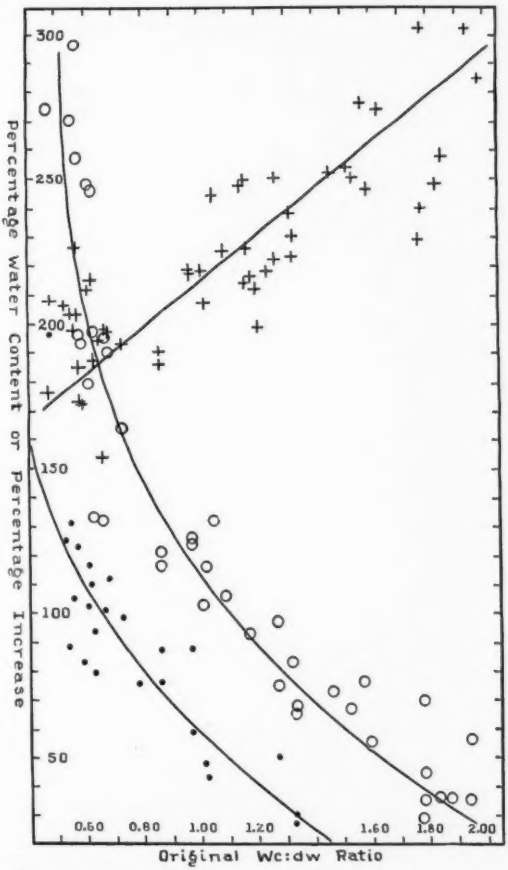

FIG. 7.—The 24 hour ("saturation") percentage water content (+) of leaves of bushes whose existing water contents ranged from 47 to 200%. For the same bushes the 24 hour *increase* (○) is shown as percentage of original value, and for some bushes the percentage increase after 1 hour (●). Curves drawn merely to indicate general regions in which the points fall.

ratio must be due principally to increase in water content; dry weight could not diminish so greatly in 15 minutes.

Further evidence in regard to how much dry weight may occur in leaves of severed shoots while in the moist chambers was obtained by the half-leaf method already described. The results of five experiments indicate that the change in water content is about eight times that of the dry weight.

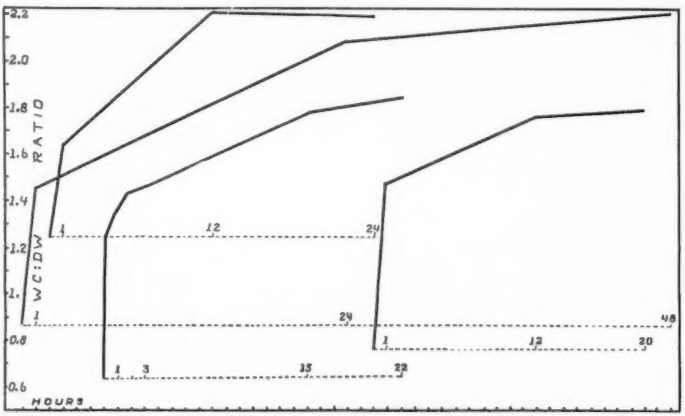

FIG. 8.—The wc:dw ratio of leaves of four shoots in a moist chamber at various time intervals after cutting from the plant and putting the stems in water.

Changes in leaves of the cut shoots during 24 hours in a moist chamber averaged 5.05% loss in dry weight

41.2 % increase in water (mg.)
46.1 % increase in wc:dw
14.5 % increase in water as percentage of fresh weight.

These experiments were conducted at the University of Cincinnati. In Arizona, higher temperatures may have led to greater change in dry weight, but probably not more than as much as to account for 15 per cent of the 24 hour increase in the ratio.[5]

EFFECT OF ELIMINATING TRANSPIRATION.—In another series of experiments with shoots inclosed by moist chambers, the stems were not cut and put in water but were left intact, the moist chambers

[5] A single test of the temperature effect in Tucson indicated no appreciable difference between 28° and 40° C. in the rate of increase of the wc:dw ratio.

being adjusted to the shoots in the field. Any increase in water content under these circumstances must be from the existing supply in the stems, roots, and soil. This type of experiment, carried out according to the procedure outlined below, proved to be a very simple and effective way of determining roughly the relative influences of transpiration and of insufficient water supply on the water balance.

A bell jar furnished with one or more wicks of paper or cloth toweling extending from the top down into a shallow pan of water is adjusted over the selected shoot. The pan of water rests on a support covered with one or more layers of waterproofed fabric which is fitted snugly around the stem of the shoot and up around the base of the bell jar, and made tight with elastic tubing. Two coats are fitted over the bell jar: one of thick paper felting for heat insulation, and an outside coating of glistening white paper to minimize sunlight absorption. The temperature within the chambers does not differ significantly from the external temperature. Dew formed in the chambers as a result of temperature fluctuation has always to be evaporated before leaves are picked for determination of wc:dw ratio. To outward appearance, shoots of *Larrea* which have been in a moist chamber even as long as 2 or 3 days appear normal in every respect. The leaves, however, doubtless suffer some loss of their substance owing to continued respiration in the absence of photosynthesis, so that the wc:dw ratio may be expected to increase even independently of any increase in water content.

In contrast to the experiments with cut shoots, no sudden large increase in water content takes place. Indeed, except in bushes k_{31}, x, y, and z (table V), the increase in the wc:dw ratio as a result of the use of moist chambers in the field was small, and in many cases may have been due to dry weight decreases.

When only one shoot of bushes t_{31}, h_{31}, and bl was inclosed by a moist chamber, similar diurnal fluctuations were shown by both exposed and experimental leaves, although the latter were protected from transpiration. This implies that protected leaves lost water during the day to nearby exposed transpiring leaves. Transpiration increases the osmotic concentration of the sap of exposed leaves over that of protected leaves so that the former can draw on the protected leaves for part of their water supply. This translocation of water

from one shoot to another indicates a deficient soil water supply, and did not occur when there was ample, as for bushes g, bs, and k31 (table V). Bushes x, y, and z (fig. 9) show both situations. By com-

TABLE V

CHANGE IN LEAF WC:DW RATIO OF INTACT SHOOTS IN A MOIST CHAMBER

Bush	Date	Number of WC:DW determinations	Variation limits of WC:DW ratio		24 hr. "sat." WC:DW	Characteristics of the WC:DW ratio of the protected leaves, etc.
			Exposed	Protected		
Ej	July 15–28 1931	32	0.57–0.61	0.58–0.66	1.70	Whole bush covered July 21; increase over control, and variations slight
Aj	July 9–13 1931	10	0.60–0.62	0.65–0.66	1.87	Readings at 3 P.M.
t31	June 26–28 1931	20	0.74–0.83	0.75–0.86	.....	Follows control
g	June 14–15 1930	10	0.74–0.83	0.90–0.93	1.93	Does not follow control; readings at 5:30 A.M. and 2:30 P.M.
bs	July 27–30 & Aug. 3–4 1930	18	0.67–0.90	0.80–1.02	.....	Does not follow control; no midday drop
h31	July 2–11 1931	18	0.83–1.02	0.94–1.07	1.77	Follows control, when not entirely covered
k31	July 1–5 1931	15	0.92–1.01	0.98–1.37	.....	Does not follow control; marked increase when entirely covered
bl	June 17–18 1931	14	0.90–1.09	0.88–1.09	2.18	Follows control
x, y, & z	Aug. 26–Sept. 5 1931	66	0.99–1.50	1.01–2.16	2.45	Follows or not, depending on conditions (see fig. 9)

parison of the curves for x and y for August 26–27 with those of all three bushes for September 1–2, the effect of rains which fell prior to September 1 is indicated. It is interesting to compare also bushes g and t31 (table V), exposed shoots of which had the same leaf water content and diurnal fluctuation, 0.74–0.83. The water content of the protected leaves of bush h31 decreased at midday just as did the exposed control, while the wc:dw ratio of protected leaves of bush g

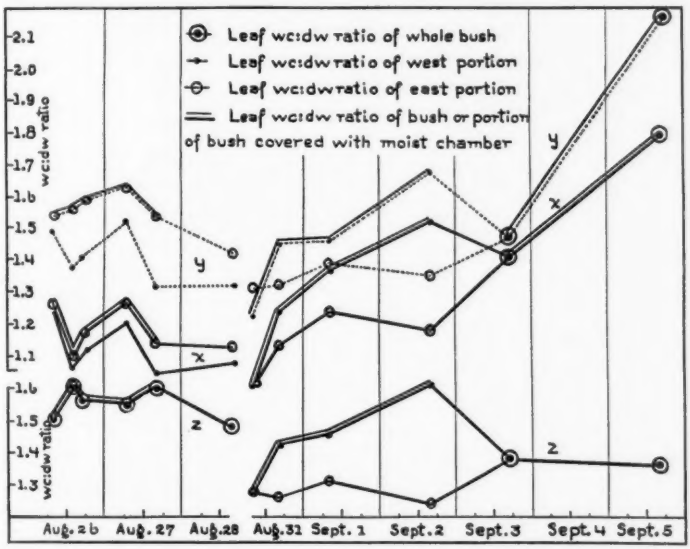

FIG. 9.—Variations in wc:dw ratio of leaves of bushes x, y, and z when partly and when completely protected from transpiration by the use of moist chambers in the field.

remained about constant or increased slightly. The wc:dw ratio of the exposed shoots alone fails to indicate the better water supply of bush g.

The effects of water translocation can be avoided by covering entire bushes. This was done for the small bushes Ej, h31, k31, x, y, and z. Even when completely covered, the resulting increase in the wc:dw ratio for bushes Ej, h31 (table V) and for bush z, Aug. 26–27 (fig. 9), was surprisingly small. The very slow reduction of the water tension, when transpiration is held at minimum by means of a moist

chamber, is convincing evidence of a deficient water supply. Transpiration, having once reduced the water content to a low level, is relatively much less important than the diminishing water supply in maintaining a low wc:dw ratio in these bushes. It is to be emphasized that this is the usual situation; not transpiration, but insufficient soil water is what maintains the low moisture content in *Larrea* leaves.

On the other hand, when there is enough water for growth, transpiration is very active in restricting the wc:dw ratio. Thus Sept. 3–5, when all of the shoots of bushes x and y (fig. 9) were covered, a comparatively rapid increase took place while bush z, entirely exposed, suffered a slight decrease. Here again the inadequacy of the wc:dw ratio of exposed leaves alone to indicate the situation is seen by comparing the data for h_{31} and k_{31} (table V). The wc:dw ratio of the leaves of both bushes is about the same. The moist chamber experiments, however, show that water is relatively more available to the leaves of bush k_{31} which when covered exhibited a markedly increased ratio.

General discussion.—The data presented in this section demonstrate that of two bushes having the same water content, one may be much further from its saturation water content or may have a significantly smaller water reserve than the other. The use of moist chambers in the field on intact plants may prove to be a valuable method of investigation of the water balance of other species.

The amount of deviation of the natural wc:dw ratio from the saturation value is related somewhat to the capacity of a plant to endure drying, but is clearly not to be taken as an indicator of this capacity. The saturation wc:dw ratio is never attained in nature; it is not a constant; and it is higher than the existing ratio partly because of dry weight changes (**39**). The inconstancy of the saturation water content is easily understood, for the total water capacity of a cell depends first upon the varying difference between its vapor tension and that of its environment, and second, upon the extensibility of its walls. The relatively high capacity shown by young leaves is no doubt due to the greater plasticity of their walls.

The great rapidity of water intake when shoots are cut under water (fig. 8) evidently indicates that a high tension normally exists

in the hydrostatic system of the shoots. The elimination of transpiration from intact shoots does not immediately relieve this high tension. The highest ratios attained by leaves on intact shoots, even though these are surrounded by a nearly saturated atmosphere, are still well below the saturation ratios (table V).

TABLE VI

DIFFERENCE BETWEEN EXISTING AND SATURATION WC:DW RATIO IN PERCENTAGE OF SATURATION VALUE

REFERENCE	REGION	HABITAT	PLANT	PERCENTAGE DIFFERENCE ("DEFICIT")
(11)	South Russia	Swamp	Alisma plantago	0.5–7.7
		Meadow	Rumex confertus	4–9
		Steppe	Plantago major	19–40
(26)	Arizona	Desert	Encelia farinosa	–50
(25)	Arizona	Encinal	Quercus emoryi	28–29
			Arctostaphyllos pungens	17–27
(37)	South Africa	Desert	Passerina spp.	–55
(31)	Egypt	Desert	Non-succulent plants	35–56
			Mesembryanthemum	19
(33)	Hungary	Alkali steppe	Achillea millefolium	4–19
			Epilobium montanum	11–16
		Baltic seacoast	Calluna vulgaris	–23
(34)	Swedish Lapland	Arctic tree limit	Shrubs and herbs	4–10
(22)	Mid-Europe	Alpine tree limit	Ericaceae spp.	"0"–30
		Shade	Mercurialis perennis	4–12
		Sun	Coronilla varia	1–33
(21)	Jerusalem	University campus	Ficus elastica	–16
			Rosmarinus officinalis	30–65
RUNYON	Arizona	Desert	Larrea tridentata	23–77

In table VI are given approximate values for the percentage difference between the existing and saturation wc:dw ratios for various plants. The methods used by the investigators have differed, so that the data are only roughly comparable. The terms water deficit and saturation deficit have often been used to designate this difference.

It is seen that *Larrea* may endure a water content further from its maximum water holding capacity than any other plant reported.

Summary

1. From various lines of evidence it is shown that the dry weight of *Larrea* leaves undergoes diurnal fluctuations when there is enough water for growth; but under the usual desert conditions the dry weight probably changes very slowly, water conditions being chiefly responsible for the fluctuations of the wc:dw ratio. However, the usual disregard of dry weight changes in literature pertaining to percentage water content, and the use of the fresh weight basis for expressing the data, have often led to erroneous interpretations.

2. The amount of moisture relative to dry weight in leaves of *Larrea* differs with age, position on the stem, season, habitat, and time of day.

3. Apart from seasonal influences, water content increases with the age of the leaves, except during rare periods of ample soil water, when the younger more plastic leaves have the higher water content.

4. During periods of growth the water content of the foliage is not lower than that of many mesophytic tree leaves: between one and two times the dry weight. During severe drought the leaves may endure a water content less than half the dry weight, a value lower than that reported for any other seed plant (except air-dry plants or plant parts).

5. Leaves at adjacent nodes, although practically indistinguishable in appearance and structure, may differ greatly in physiological capacity, depending upon the water conditions existing during their development. Three leaf types, *a*, *b*, and *c*, may be recognized. The *a* type, most abundant, has a comparatively high water content, and very little or no drought resistance. The *b* type is intermediate in all respects to *a* and *c*. The *c* leaves are resistant to drought, have a lower water content during their expansion from the bud, and a longer life span than the *a* leaves.

6. Development during a time of water shortage seems to engender drought enduring capacity, while attainment of maximum size and water content entails loss of this capacity.

7. The drought enduring *c* leaves have two periods of enlargement,

separated by a season of drought-induced dormancy. This dormancy is not complete, as is shown by the facts that the leaves are not air-dry and that a slight diurnal variation of the wc:dw ratio occurs.

8. In general, osmotic changes follow in a reciprocal manner the diurnal water content changes, but the latter may disclose differences not evident in the osmotic measurements alone.

9. Large and very rapid increases in the water content of the leaf occur when the stems are cut and allowed to remain under water, the leaves being inclosed by a moist chamber. This implies a high tension in the tracheal sap.

10. The saturation water content is higher above the naturally existing water content for *Larrea* leaves (*b* and *c* types) than for any other species for which data have been found in the literature.

11. By following the changes of the wc:dw ratio of leaves of bushes partly or completely covered with moist chambers, the adequacy of the water supply to the leaves may be judged.

12. Under usual conditions a deficient water supply rather than transpiration is the crucial factor limiting the moisture content; while after rains the rate of transpiration may be very high and the chief cause of the considerable difference between the saturation and existing water contents.

I wish to express my gratitude to those who have criticized the manuscript of this paper, to Dr. FORREST SHREVE, who is in charge of the Desert Laboratory, and to Dr. T. D. MALLERY.

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GROWTH OF FRAGMENTS OF EXCISED ROOT TIPS[1]

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(WITH SIXTEEN FIGURES)

The experiments on the cultivation of fragments of root tips described in this paper were undertaken with two particular objectives in mind: first, to determine what types of fragments of root tips grow in pure culture; second, to observe the development of these fragments in the hope of learning by experiment something of the controlling factors.

Materials and methods

In the major portion of the experiments, root tips of corn (*Zea mays*) were used. Some experiments were performed also with root tips of wheat (*Triticum vulgare*) and of peas (*Pisum sativum*). The methods used in cultivating the root tips under sterile conditions were similar to those employed by ROBBINS (**7, 8**) and by ROBBINS and MANEVAL (**9, 10**). In securing the root fragments, the grains or seeds were surface sterilized with calcium hypochlorite or with mercuric chloride in 50 per cent alcohol, washed with sterile water, and germinated on sterile 1 per cent water agar in petri dishes. When the roots had reached a length of 1–2 cm., pieces were cut by a sterile knife and transferred to a sterile medium containing mineral salts, 2 per cent glucose, and 0.75 per cent agar in 150 cc. Erlenmeyer flasks or in petri dishes. The knives used were made by fixing fragments of safety razor blades or suitably ground sewing needles in an aluminum handle. The roots were fragmented for the most part without visual aids. In some cases a binocular microscope was used. Contaminations were few.

Three agar media were used. Medium I contained calcium nitrate, 50 p.p.m.; magnesium sulphate, 10 p.p.m.; potassium dihy-

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drogen phosphate, 10 p.p.m.; ferric chloride, 1 p.p.m.; glucose, 2 per cent; agar, 0.75 per cent. Medium II was medium I containing, in addition to the constituents given, sodium borate, 0.1 p.p.m.; manganese chloride, 0.1 p.p.m.; zinc chloride, 0.1 p.p.m. Medium III was prepared by placing half a sterile corn grain which had germinated three days at 28° C. in the center of a plate containing agar medium II. The freshly cut surface of the grain was pressed into the agar. The plates were then placed in the dark for five days to allow diffusion from the germinated grain to occur. At the end of five days the half grain was removed and the fragment of the root tip was placed on the agar plate.

Preliminary experiments

Grains of Longfellow flint corn were germinated under sterile conditions. A terminal piece of the root tip less than 3 mm. in length was removed by a cut across the root. Although the cuts were intended to be perpendicular to the long axis of the root they were in some cases more or less oblique. These short tips were transferred to a sterile agar medium in petri dishes and incubated in the dark at room temperature. Using a compound microscope and ocular micrometer, the original length of the fragment was measured. Daily measurements of growth were made and final lengths and observations were taken after 7 to 18 days.

The original length of the tips ranged from 0.27 to 2.62 mm. The shortest root tip which grew was originally 0.32 mm. long and reached on medium II a length of 3.8 mm. in 7 days. Most of the tips less than 0.4 mm. in length did not grow, however, only two out of eleven less than 0.4 mm. increasing in length. The greater proportion of those tips from 0.4 to 0.49 mm. long also failed to grow; 19 out of 50 in this group growing. The best growth on medium II in this group was a root tip originally 0.45 mm. long which grew to 15 mm. in 10 days. When the original length of the root tip was 0.5 mm. or more, the majority of the tips grew. From table I it is obvious that excised corn root tips under the conditions of these experiments must be more than 0.5 mm. in length for growth to occur in the majority of cases; but to secure growth in all cases the tips should be 0.9 mm. or more in length.

No difference in the effects of the three media on the percentage of root tips which grew in the different groups was noted. For final lengths medium III was superior to medium II and medium II was superior to medium I. A discussion of the effect of these media on the amount of growth will be given in a separate publication.

Corn root tips such as were used in these experiments consist of two main parts, the root cap and the meristem. How much of a particular excised root tip is composed of root cap cannot be stated ex-

TABLE I

PERCENTAGE OF ROOT TIPS OF LONGFELLOW FLINT CORN WHICH GREW ON AGAR MEDIUM; ROOT TIPS GROUPED ACCORDING TO ORIGINAL LENGTHS

ORIGINAL LENGTH (MM.)	NUMBER OF ROOT TIPS	PERCENTAGE WHICH GREW
0.20–0.29	3	0
0.30–0.39	8	25
0.40–0.49	50	38
0.50–0.59	31	86
0.60–0.69	44	93
0.70–0.79	25	84
0.80–0.89	30	96
0.90–0.99	3	100
1.00–1.99	8	100
2.00–2.99	84	100

actly. Microscopic examination of the excised tips on the agar medium in the culture dishes does not permit accurate measurement of the root cap as distinct from the meristem, and all measurements given are "over all" lengths. The length of the root cap varies with the individual root and is affected by the root cap cells pushed away in manipulation. Measurements of prepared sections of 15 corn root tips similar to those used in these experiments showed the root cap to vary in length from 0.26 to 0.38 mm., averaging 0.33 mm. The failure of a considerable proportion of those root tips less than 0.5 mm. in length to grow does not mean, therefore, that a piece of the root meristem 0.5 mm. or more in length is necessary to secure growth. If the shortest fragment which grew (0.32 mm.) had a root cap equal in length to the shortest root cap measured, it would contain a meristem 0.06 mm. long.

In figure 1 the percentage of root tips which grew is plotted against the original length of the tips. On the same figure a root tip is drawn showing the average length of the root cap. If averages only are considered, the average length of the meristem when 50 per cent of the root tip fragments grew was 0.15 mm., and when 75 per cent of them grew it was 0.19 mm.

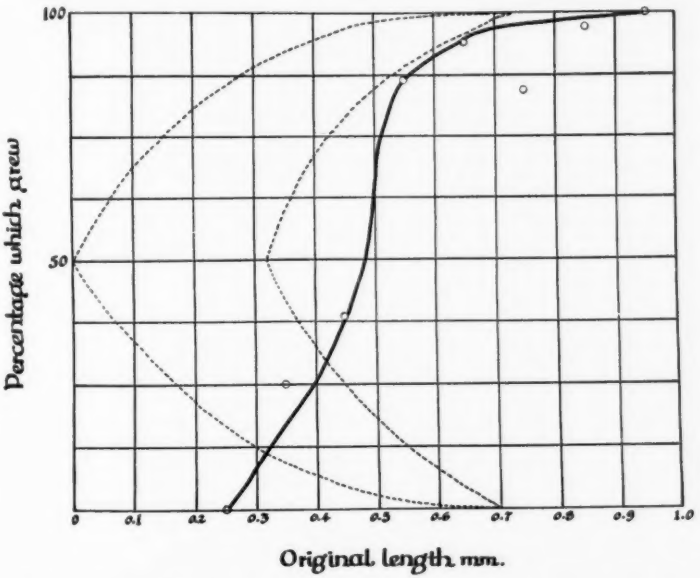


FIG. 1.—Relation between original lengths of excised root tips of corn and percentage which grew under sterile conditions on nutrient medium. Dotted lines show average length of root cap.

As growth occurs in a root when attached to the plant, the cells at the apex of the meristem remain embryonic while the older parts of the meristem differentiate in an orderly manner into the epidermis, cortex, and stele. In these experiments, in which isolated pieces of root meristem were grown, tips originally 1 mm. or more in length developed into normal roots; and most of those less than 1 mm. long, which increased in length, also formed normal roots. At the end of the experiment, the isolated piece of root meristem had formed a

root consisting of a root cap, apical meristem, epidermis, cortex, and stele. Root hairs and branch roots had been produced.

While it is not possible from these experiments to state categorically the minimum length of meristem in an excised corn root tip necessary for normal development, the following tentative conclusions may be offered. It appears that the excised root cap including the calyptrogen does not under the conditions of these experiments form a normal root. It is probable that a terminal piece of the meristem 0.1 mm. in length will form a normal root.

Normal polarity was evidenced by excised corn root tips 0.5 mm. or less in length. These tips measured less in the direction of the long axis of the root than they did in a direction across the root, and were composed of the root cap and a bit of the apical meristem. In such pieces growth occurred in the direction of the long axis of the original root and the cells elongated in the same direction as they do in the normal root.

An occasional root tip less than 1 mm. long developed into a root, normal in all respects except that the apical meristem did not remain embryonic but differentiated into elongated parenchyma cells. In such cases the tip of the root contained no embryonic cells at the termination of the experiment (fig. 2).

Furthermore, occasionally the shorter pieces of meristem did not differentiate epidermis, cortex, and stele, but developed into a flat filament (sometimes two) the cells of which resembled cortical and epidermal cells. Such filaments lacked a stele and embryonic region and were usually tightly coiled.

Many of the shorter root tips, especially those less than 0.5 mm. in length, did not elongate but formed a mass of cells, circular in outline, on the surface of the agar. The diameter of this mass was rarely more than two or three times the original length of the tip. The mass consisted of a central core of cells surrounded by a jelly-like matrix in which root cap cells were imbedded. In some cases the original tip consisted of root cap only, in others a bit of the meristem also was included. A typical development of this type is shown in figure 3. No information as to whether cell division occurred in the formation of these masses is at hand. It is probable that they resulted from the enlargement of individual cells and the gelatinization of cell walls.

FIG. 2

FIG. 3

FIGS. 2, 3.—Fig. 2, tip of developing excised corn root tip originally 0.48 mm. in length. Some root cap cells show an extrusion of protoplasm. Note that tip is not meristematic but is composed of elongated parenchyma cells. Fig. 3, development of excised corn root tip originally 0.48 mm. long. Note central core composed largely of root cap cells and scattered root cap cells imbedded in jelly-like matrix.

Microscopic examination showed that the outer layers of the cell walls of the root cap cells had gelatinized. This gelatinization probably does not include the middle lamella, as bits of a delicate network were visible here and there in the gelatinous mass. These were interpreted as middle lamellae left when adjacent parts of the cell wall gelatinized. The root cap cells do not migrate but are entirely passive in their separation one from another, which is due to the swelling of the gelatinized cell wall layers between them. This is probably the explanation for the "migration" of root cells reported by CHAMBERS (1). The separated cells frequently became half-moon shaped and an extrusion of protoplasm was often observed on the convex side of the cell, presumably through a pore formed in the wall (fig. 2).

Why cells at the apex of the root meristem should retain their embryonic character, while a short distance from the apex they lose it and form the various permanent tissues of the root, is a question for which as yet we have no adequate answer. What are the conditions which determine the existence of a meristem and conversely what determines the differentiation of cells? In the preliminary experiments just described, some of the short pieces of the meristem did not retain their meristematic character, although all the tissues normally formed in a root were differentiated; and other pieces did not form all the permanent tissues normally found in the root. Why should pieces of the apical meristem of a corn root tip respond in these fundamentally different ways? Are the differences in their responses determined by the treatment of the fragment (injury from cutting, culture medium), or by the amount or kind of tissue included?

The preceding experiments were concerned with short root tips which had been removed by a cut across the root, perpendicular to the long axis of the root in most cases but oblique in some. When a tip 0.5–1 mm. in length is removed from a corn root by a transverse cut, the excised piece includes the initial cells at the tip of the embryonic region and a portion of the three histogens present in the meristem (plerome, periblem, and dermatogen). If growth took place it might be anticipated, since the histogens from which the various permanent tissues develop are all present, that a complete

root with a differentiated stele, cortex, and epidermis would be formed. Even in such a case, however, it is possible that too small a piece of the apical meristem would not grow under the conditions of the experiment, or, if it did, all of it would differentiate and no embryonic region would remain at the end of the experiment.

When a root tip is cut obliquely, the fragment removed may or may not include the initial cells and may lack one or more of the histogens. The development of such fragments might conceivably be very different from that of a bit of the meristem which included the initial cells and some of each of the three histogens.

To explore the relation which might exist between the character of the tissues included in a root tip fragment and its development, root tips 1–2 mm. in length were fragmented by longitudinal, oblique, or transverse cuts and the fragments cultivated on a nutrient medium. To cut fragments from a seedling root tip 1–2 mm. in length which had previously been removed from the root was found to be extremely difficult. Fragmentation was simplified by making the proper cuts before the tip was removed from the root. After fragmentation, the pieces were placed on medium I in petri dishes or in 150 cc. Erlenmeyer flasks and grown in the dark. After one to three weeks, the resulting structure was transferred to a drop of lactophenol containing cotton blue and acid fuchsin (4). The cleared root fragments, in which differentiated cells stained red and embryonic cells a deep blue, were then examined microscopically. The results of the experiments to be described show that a fragment of the apical meristem developed normally if it included a portion of the plerome not less than 0.1–0.2 mm. in length and equivalent to between one-quarter and one-half of that found at the root apex. If less than this amount of the plerome was included or if periblem and dermatogen alone were used, a complete root was not formed.

Further experiments

Root tips fragmented by median longitudinal cuts

Nine root tips of corn, varying from 1 to 2 mm. in length, were cut longitudinally into two parts, nearly equal in size. Both pieces of six of these sets of fragments developed into complete normal roots. In one week the pieces originally 1–2 mm. long grew to lengths of 16–18

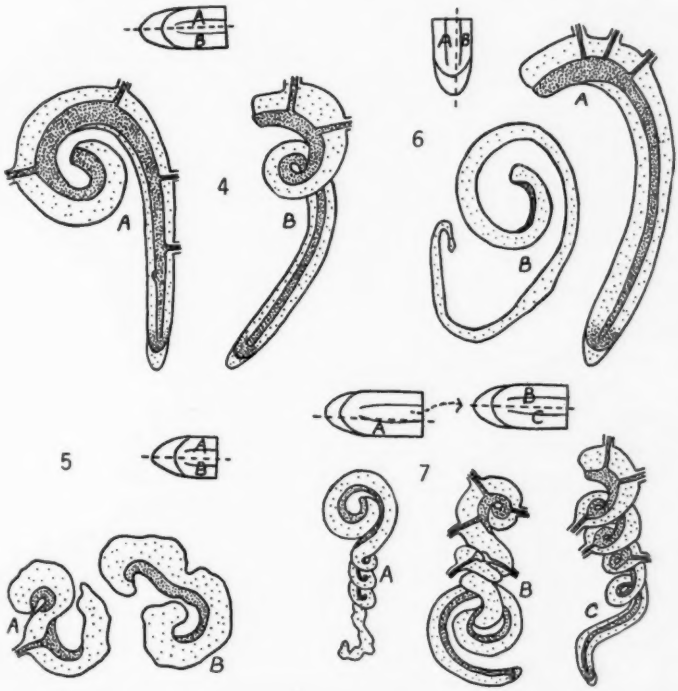
mm. and each produced several secondary roots. Such a set of fragments is illustrated in figure 4. In the upper part of the figure is a diagram showing how the tip, measuring 1.2 mm., was cut into two equal pieces, *A* and *B*. The development of these two pieces is shown diagrammatically in the lower part of the figure at *A* and *B*. The base of each of the developed fragments contains stele (shaded) on one side but no cortex; toward the tip the stele is more nearly central, until eventually the root is entirely normal.

In the original half root tip the plerome is exposed on one side. This exposure in this way amounts to changing its relative position in the root, since in the half root it no longer occupies a central position. If the relative position of the plerome in the original root is a factor of importance in determining its differentiation into the elements of the stele, we might expect that the exposed plerome in a half root tip, formed by a medium longitudinal cut, would differentiate into epidermis and cortex instead of into stele. However, in the basal portions of the roots which developed from these half root tips the stelar tissue was located on one side in the position occupied by the plerome in the original half root (fig. 4). The change in relative position had no noticeable effect upon the differentiation of the basal and older portion of the plerome.

On the other hand, continued growth resulted in the formation of a complete root, not a half root. This indicates that cells at the tip of the embryonic region are not fixed in the potentialities of their development, as are the older cells of the plerome, but are capable of development into any of the various elements of the root. It is probable that this group of cells is not limited to the initial cells as it does not seem possible that a razor, in dividing a root tip into halves, would separate the group of initial cells into two groups so that each half of a root tip would possess some of them. In any event, it is clear that the fate of the plerome cells is fixed early in their development in such fashion that change in relative position does not materially modify their development. Just how early determination occurs cannot be stated, but judging from the length of the basal portion of the root which contains stele on the outside, it would appear to occur within 0.2 or 0.3 mm. of the apex of the root meristem.

Of the pairs of fragments which did not develop into normal

roots, the members of one pair had increased in length from 1 to 8 mm. in five days. Some stele was formed, but at the end of the experiment no apical meristem was present in either piece; all of the



FIGS. 4–7.—Fig. 4, development of fragments of corn root tip originally 1.2 mm. long and cut longitudinally into equal parts; each piece developed into a normal root but basal portion has stele on one side. Fig. 5, development of fragments of corn root tip originally 1.0 mm. long and cut longitudinally into equal parts by a coarse razor (Gitsnife). Neither piece developed into a normal root although stelar tissue was differentiated and branch roots formed. Fig. 6, development of fragments of corn root tip originally 1.0 mm. long and cut longitudinally into unequal parts. Larger piece developed into a normal root although with stele on one side at the base; smaller piece formed some stele at base but lacked root cap and apical meristem. Fig. 7, development of fragments of corn root tip originally 2.0 mm. long and cut longitudinally into unequal parts, the larger piece then cut longitudinally into equal parts as shown in diagram. *A* formed some stele but lacked root cap and apical meristem; *B* and *C* developed into normal roots although with stele on one side at base.

meristem had differentiated into elongated parenchyma cells. This set of fragments is shown diagrammatically in figure 5.

In another pair, the root fragments were grown for two weeks and the lengths of the pieces increased from 1 mm. each to 8 and 16 mm. respectively. Each piece contained some stele. Neither piece possessed an apical meristem.

Another set of fragments grew from 1.5 to approximately 15 mm. Both were tightly coiled, the cut surface forming the inside of the coil and no apical meristem was present.

These three sets of fragments in which all of the meristem differentiated were prepared by using a "Gitsnife" instead of the thinner blade of the Gillette type which was used in the other experiments. It is probable that their differentiation was the result of the greater injury from the thicker knife. The complete differentiation of these fragments cut with the coarser knife may have been due to the influence of by-products from injured cells or to the actual destruction of cells at the apex of the meristem. WHITE (13) has called attention to the effect of injury on the growth of excised wheat root tips.

The results secured with excised corn root tips split longitudinally into two equal parts are similar to those observed by LOPRIORE (2, 3), SIMON (11), and NEMEC (5) on the regeneration of split roots attached to the grain or seed. SIMON found that if root tips of corn are split longitudinally for 1 cm. from the tip regeneration occurs through the activity of cells at the apex of the meristem where little or no differentiation is evident; farther back from the tip a partial regeneration takes place; and approximately 1 mm. from the end of the original root tip, only scar tissue forms over the cut surface.

Pea roots responded in the same fashion as did corn although the structure of the tip of the pea root is not the same as that of corn. Four root tips of pea were divided into equal parts by a longitudinal cut. Each of the halves developed into normal roots.

One root tip only of wheat was divided longitudinally into equal halves. The original tip was 1.5 mm. long. At the end of one week's growth each piece was 5–6 mm. long and coiled, especially at the base, with the cut surface toward the inside of the coil. The base of each fragment for 1–2 mm. was made up of large nearly cubical cortical cells and epidermal cells, 50–55 μ wide, and on one side

stelar tissue with xylem vessels had formed. Numerous root hairs were present except on the side composed of stele. Near the tip the epidermal and cortical cells were narrower and longer, 100–130 by 18 μ; the stele, still one side, did not show fully differentiated xylem vessels. The tip, surrounded by root cap cells, consisted of elongated parenchyma cells which were not embryonic. The tip was six or eight cells in width. What appeared to be the shrunken remains of dead cells were present on the inside of the coil for about 2 mm. from the base.

Evidently the cells in the half wheat root tip underwent little or no division, but differentiated, those at the tip of the meristem elongating but forming none of the specialized cells of the older portion of the root.

Root tips cut longitudinally into unequal parts

Eighteen root tips of corn 1–2 mm. in length were cut longitudinally with one portion larger than the other. These fragments were allowed to grow for one to three weeks. In every case the larger portion developed into a normal root with an apical meristem. All of the smaller portion differentiated. At the end of the period of growth it contained no apical meristem, and, except for a small amount of stele at the older and basal part of some of the fragments, consisted entirely of cortical and epidermal cells. A typical example of this sort of development is shown in figure 6.

When the smaller portion contained no plerome it elongated more rapidly than the larger piece, developed no root hairs, and completed its growth in two or three days. In a specific case the smaller fragment consisted of a thin slice from one side of the root tip. At the end of two days it was 8 mm. in length and no root hairs were evident. At the end of 18 days it was still 8 mm. in length and consisted of a thin filament of cortical and epidermal cells with no apical meristem and no root hairs. The larger fragment at the end of two days was 6 mm. long, slightly coiled toward the side with the plerome exposed, and had an abundance of root hairs. At the end of 18 days it was 27 mm. in length and for the major portion of its length was a normal root.

The development of the larger fragment of a corn root tip divided

longitudinally into unequal parts agrees with the results observed with fragments cut into equal parts. If each of the equal halves develops into a complete root, a fragment formed by a longitudinal cut and composed of more than half the root should develop in the same way.

The smaller fragments, in which all of the meristem differentiated, consisted of dermatogen and periblem and in some cases a small part of the plerome. Even though these fragments contained part of the plerome, they lacked the initial cells and the cells of the adjacent histogens. From an examination of prepared sections of corn root tips it was estimated that the smaller fragments lacked the tip cells of the plerome, periblem, and dermatogen for a distance of 0.15–0.2 mm. from the apex of the root meristem. The differentiation of these smaller fragments shows that those cells of the meristem which are within 0.15–0.2 mm. of the apex have totipotentialities. Cells more distant from the apex are, under the conditions of these experiments, fixed in the possibilities of their development. Further, it is evident that exposing the periblem cells does not materially affect the character of their differentiation.

The more rapid elongation of the smaller fragments which developed no stele is probably due to a greater effective internal pressure in the cells of the dermatogen and periblem than in the plerome. That such a pressure exists in the cells of the former two tissues is confirmed by the coiling of the half root, which always occurred toward the cut side. Whether this greater pressure is the result of a greater actual pressure within the cells or because of thinner and more pliable cell walls in the periblem and dermatogen, cannot be stated from these observations. The failure of the pieces composed of periblem and dermatogen to produce root hairs, in contrast to those pieces which included plerome also and which elongated more slowly but formed many root hairs, is probably explained as follows:

A piece of root which contains plerome elongates less rapidly than a piece which lacks plerome. In the latter case the pressure from within an epidermal cell against the cell wall is relieved by the stretching of the entire cell, which is possible because no plerome differentiating into stele is present to limit the stretching of the cell. Where plerome is present in the original piece, the internal pressure

in the dermatogen or the epidermis is not relieved by the stretching of the entire cell and remains sufficiently high to push out a root hair.

The results secured with pea roots were similar to those found for corn. Three root tips of pea were cut longitudinally so that one piece was noticeably larger than the other. The smaller portions contained little or no plerome. The larger fragment of each set developed into a complete root, the smaller elongated somewhat but had no apical meristem at the end of one week's development.

On the other hand, neither piece of the single wheat root tip which was cut developed into a normal root. One root tip was cut longitudinally into unequal pieces. The original tip was 1 mm. in length, and after one week the smaller fragment, *A*, was 4 mm. long and the larger, *B*, was 5 mm. long. Neither piece formed a normal root. *A* consisted of epidermal, cortical, and root cap cells with no stele and no root hairs. The apical meristem had differentiated into elongated parenchyma cells. *B* produced one branch root; stelar tissue was present but no apical meristem could be found. The stele extended almost to the tip of the root, which was composed of cortex and epidermis-like cells. Root hairs were numerous and were present over the tip of the fragment.

Fragments formed by two or more longitudinal cuts

Fourteen excised corn root tips 1–2 mm. in length were divided into three or more fragments by longitudinal cuts. In four sets of such fragments no terminal meristem was present after one week of development. All of the apical meristem had differentiated. In all others the largest fragment grew and formed a normal cylindrical root with a terminal meristem. Even though strips of periblem and dermatogen were cut from as many as three sides of the tip, if half or more than half of the plerome remained the piece developed into a normal cylindrical root. When the root tip was divided longitudinally less than half of it was sufficient in these experiments for the production of a normal root, but a quarter of the root tip did not form a normal root.

Two examples are illustrated in figures 7 and 8. In figure 7 the results are shown for a root tip which was divided longitudinally into two unequal parts. The larger part was divided by a longitudinal

cut perpendicular to the cut surface into two equal parts, *B* and *C*. After one week the smaller fragment, *A*, of the original tip was 6 mm. long and completely differentiated. *B* and *C* were each 12 mm. long and had developed into normal roots.

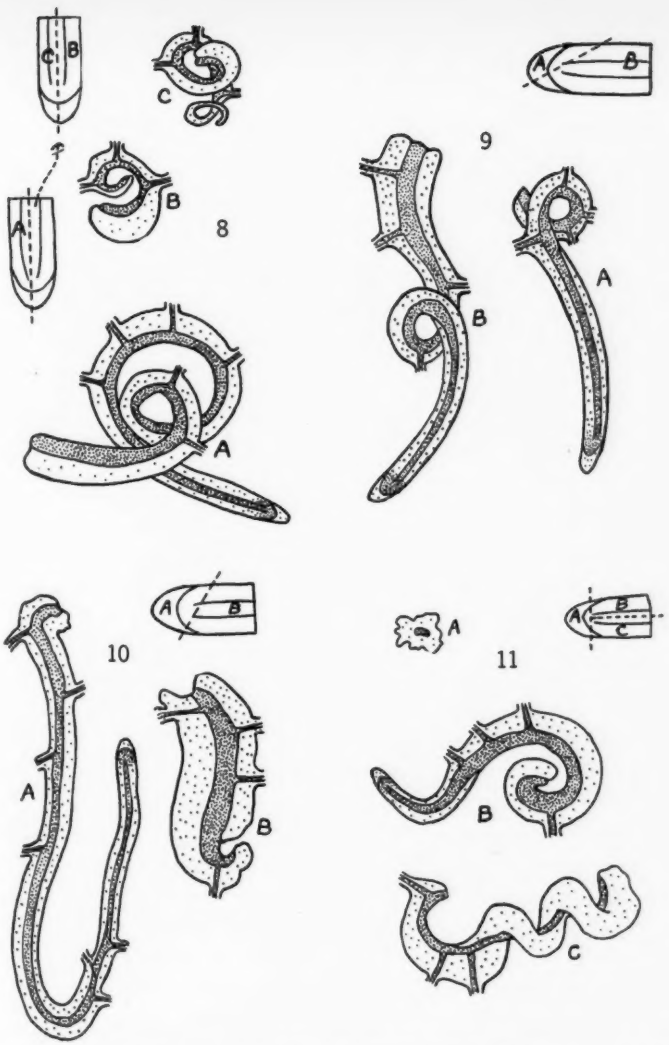
In the experiment illustrated in figure 8 the original root tip was divided longitudinally into two equal halves and one of the halves was cut again into equal parts, *B* and *C*. *A* represented half the root tip, *B* and *C*, one quarter of it. After one week *A* was 21 mm. long and had formed a complete root. *B* and *C* were each 4 mm. long and both had differentiated completely. It is interesting to note that even in the quarter root tips the pericycle functioned normally and formed branch roots.

Two root tips of pea were each cut into three fragments by longitudinal cuts. The results were similar to those secured with corn. A piece which was less than half but more than one quarter of the root developed into a normal root tip. A root tip 2.2 mm. in length was divided unequally by a longitudinal cut into piece *A* and a larger piece. The larger piece was divided equally into *B* and *C*. At the end of one week *B* and *C* each possessed normal root tips while *A* lacked one.

From the experiments on the growth of fragments of root tips formed by longitudinal cuts, it is evident that all parts of the apical meristem are not equally important in determining the tissues which are differentiated and the persistence of an embryonic region.

Pieces which did not include the apex of the plerome did not form normal roots even though they included meristem from the dermatogen and periblem to within an estimated 0.15 or 0.2 mm. of the apex of the meristem. While this statement emphasizes the importance of the apex cells of the plerome for the differentiation of a normal root and for the persistence of the apical embryonic region, the apex cells of the dermatogen and periblem may be equally important since they also were included in the fragments which developed normally. The initial cells, however, are not necessary for normal development.

Quantitative relations also are important since normal development did not occur even if apex cells of the plerome were included in



FIGS. 8–11.—Fig. 8, development of fragments of corn root tip originally 2.0 mm long and cut longitudinally into equal parts, one of these then cut longitudinally into equal parts. Half fragment developed into a normal root; quarter fragments lacked root cap and apical meristem. Fig. 9, development of fragments of corn root tip originally 2.0 mm. long and cut obliquely through apex of meristem; the terminal fragment measured 0.7 mm. in length. Both pieces formed normal roots. Fig. 10, development of fragments of corn root tip originally 1.3 mm. long and cut obliquely back of apex of meristem. Terminal piece formed a normal root; basal piece differentiated but lacked root cap and apical meristem. Fig. 11, development of fragments of corn root tip originally 1.5 mm. long and cut as shown in diagram. Terminal piece was 0.5 mm. long and formed an unorganized mass; the larger piece, *B*, developed a normal root and the smaller piece, *C*, lacked root cap and apical meristem.

the fragment, unless (in corn and pea) more than one fourth of the group was present.

Furthermore the treatment of the fragment is concerned, as is illustrated by the failure of corn root tips to develop into normal roots if they were cut longitudinally with a thick razor blade. In this case the significant fact is that the group of cells which normally remains embryonic lost its embryonic character and differentiated.

The number of wheat root tips cut longitudinally is too small to permit dogmatic conclusions. Nevertheless the results, which contrast with those secured with corn and pea, are of interest. The smaller size of the wheat root tips and the smaller quantity of meristem in a longitudinal section of a tip may suggest the explanation for the failure of such fragments to form complete roots. A second possibility is that the longitudinal cut destroyed certain apical meristematic cells necessary for the continued development of meristem.

Fragments formed by oblique cuts

Twelve root tips of corn were cut obliquely, with the cut dividing the embryonic region into two parts. In four of the tips the cut was made as nearly as possible through the apex of the meristem. Both fragments from two of these tips developed into normal roots. The results in one case are illustrated in figure 9. The original root tip was 2 mm. in length. The smaller fragment, *A*, measured approximately 0.7 mm. on the longer side. After one week *A* was 15 mm. in length, *B* was 20 mm., and the terminal portion of each was a normal root with apical meristem.

The fragments of the other two root tips cut in this manner elongated somewhat. The tip portions formed thin filaments of parenchyma cells. The basal portions produced root hairs and branch roots. No apical meristem was present.

Eight of the root tips were cut obliquely through the apical meristem but back of its apex 0.5–0.75 mm. from the end of the root and more nearly transverse. Without exception the tip fragment in each set developed into a normal root and the basal fragment differentiated but lacked an apical meristem at the end of the experiment. One such experiment is illustrated in figure 10. After 15 days the tip portion, *A*, was 30 mm. long and a normal root. The basal portion,

B, was 8 mm. long, and while it had normal branch roots, no apical meristem was present.

One root tip of corn was cut diagonally through the basal portion back of the apical meristem. The tip fragment, originally 1.9 mm. on the longer side, developed into a normal root. The rear fragment in addition to numerous branch roots formed a slender normal root which apparently developed from the cut surface. It is probable that true regeneration was not involved in this case but that the development of a branch root occurred as described by SIMON (11) for decapitated roots attached to the grain.

When oblique cuts were made through the apex of the meristem, both pieces of a set of fragments formed complete roots in two cases and neither piece formed complete roots in two additional cases. Why should this be? We cannot define the exact line of the cut when oblique cuts were made through the apex of the meristem nor say exactly what cells were included in each of the two fragments formed. However, when both pieces formed complete roots sufficient meristem must have been included in the terminal piece for normal development. Judging from the preliminary experiments in which most of the fragments were removed by transverse cuts, the terminal piece probably included cells for a distance of 0.1 mm. from the apex of the meristem. This estimate is supported by diagramming a root tip and noting where an oblique cut through the apex might run. When neither piece developed a complete root the cut may have been more nearly through the apex of the meristem, forming a terminal piece with too little meristem to permit development. In this case, however, there is no obvious reason why the basal portion should not develop normally. It is possible that failure was due to injury from the cut, as appeared to be the case in the three sets of fragments cut longitudinally with a Gitsnife.

One pea root tip 2 mm. in length was cut diagonally through the meristem. Each piece at the end of one week had produced a normal root.

Five root tips of wheat were cut diagonally through the apex of the meristem. None of these fragments developed into normal roots. The tip fragments at the end of the period of growth consisted of masses of root cap cells and filaments of parenchyma cells. The bas-

al portions elongated somewhat and differentiated stele, cortex, and epidermis with root hairs but formed no apical meristem. An occasional branch root was formed by the basal pieces.

Nine root tips of wheat approximately 2 mm. in length were cut diagonally through the basal portion of the apical meristem, the tip fragments measuring 0.7–1.1 mm. on the longer side. All the tip fragments except one continued development and formed normal roots. The one exception was torn in cutting. The basal fragments, except the one which was torn, produced thin normal roots which developed from the plerome and initiated development a short distance back of the cut surface.

The regeneration of the apical meristem and root cap by the basal fragments of wheat root tips cut diagonally just back of the apical meristem is similar to that described for decapitated roots of corn and other plants by SIMON (**11**) and NEMEC (**5**).

FRAGMENTS FORMED BY TRANSVERSE CUTS

In addition to the preliminary experiments in which corn root tips were fragmented by transverse cuts, a few observations were made on wheat.

Two root tips of wheat were cut transversely through the embryonic region. The tip fragments measured 0.3 and 0.45 mm. respectively; the basal fragments were 2 and 1.45 mm. in length. None of these pieces developed into normal roots. After one week the tip portions were masses of root cap and parenchyma cells, with some of the parenchyma cells organized as filaments. The basal fragments differentiated a stele and produced branch roots but had no apical meristem. These results are similar to those in which oblique cuts were made through the apex of the meristem. WHITE (**13**) secured development of wheat root tips (with root cap removed) less than 0.1 mm. in length.

Three root tips of wheat were cut transversely through the base of the apical meristem, the tip fragments measuring about 0.75 mm. in length. Two of the tip fragments formed normal roots; one formed a mass of cells. The basal fragments of two of the root tips elongated to twice their original length and formed stele but developed no apical meristem. The basal piece of one of the sets of fragments in

which the tip portion grew normally showed at the end of two days the beginning of the regeneration of an apical meristem.

Fragments formed by combination of longitudinal, transverse, and oblique cuts

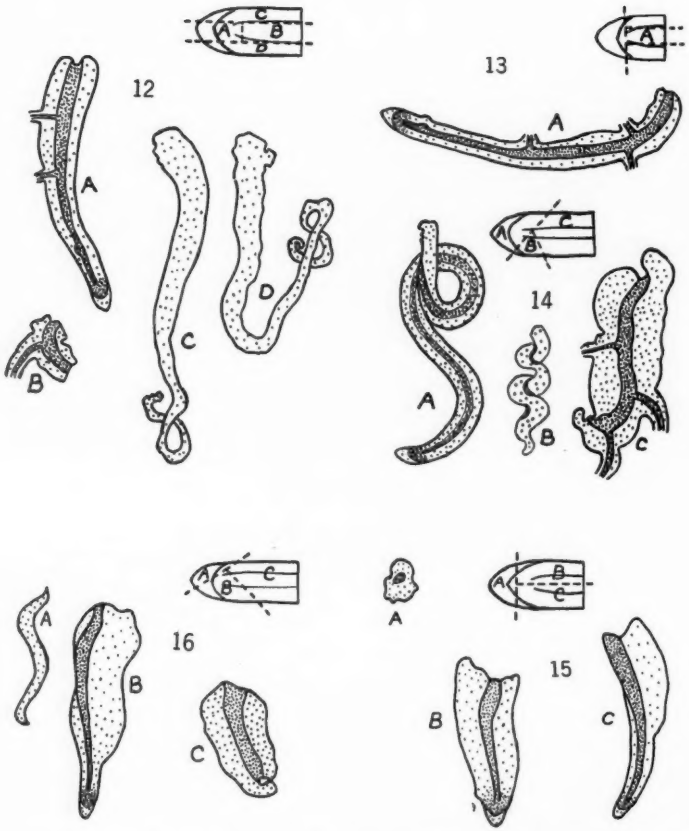
In addition to the experiments described, a few root tips were fragmented into three or more pieces by various types of cuts.

A corn root tip (fig. 11) was cut transversely so as to include the very tip of the embryonic region in the terminal piece, *A*. The basal portion was then divided longitudinally into two unequal parts; a larger, *B*, and a smaller, *C*. The tip fragment showed little development and after a week consisted of a small gelatinous mound with a central core of cells surrounded by loose root cap cells. *B* developed into a normal root and *C* increased in length and formed branch roots but had no apical meristem. *B* and *C* each measured 7 mm. at the end of one week.

From another tip (fig. 12) two side portions, *C* and *D*, were removed and the remaining portion was divided into two pieces by a transverse cut well up in the embryonic region. The tip portion, *A*, contained part of the root cap and the apex of the meristem, the rear portion, *B*, contained part of the meristem but not its apex. The side fragments, *C* and *D*, which were cut so as to avoid including any of the plerome, elongated from 1.1 to 12 and 14 mm. in seven days, and consisted of a filament of cortical and epidermal cells. No apical meristem was present. The tip fragment, *A*, elongated from approximately 0.6 to 9 mm. and formed an entirely normal root. The basal fragment, *B*, differentiated a stele and formed branch roots but no apical meristem.

In figure 13 the development of a central piece of a corn root tip is illustrated. The original root tip was approximately 1.4 mm. in length. A transverse cut was made just back of the attachment of the root cap. Longitudinal cuts were made on two sides of the remainder of the root tip. These cuts were designed to include a small amount of the plerome. The central piece only was grown. After one week the resulting structure was 11 mm. long and had formed a normal tip with apical meristem as well as branch roots.

Two root tips were cut as shown in figure 14. In each the tip frag-



FIGS. 12–16.—Fig. 12, development of fragments of corn root tip originally 1.1 mm. long and cut as shown in diagram. Fig. 13, development of central fragment (0.8 mm. long) of corn root tip cut as indicated in diagram. Fig. 14, development of fragments of corn root tip originally 1.5 mm. long and cut as shown in diagram. Terminal piece, *A*, developed into a normal root; *B* and *C* lacked root cap and apical meristem. Fig. 15, development of fragments of pea root tip originally 2.5 mm. long and cut as shown in diagram. Terminal piece 0.5 mm. long; basal piece cut into equal parts. Fig. 16, development of fragments of pea root tip originally 1.8 mm. long and cut as shown in diagram. *A* developed into filament of cortical and epidermal cells; *B* formed normal root; *C* lacked root cap and apical meristem.

ment, *A*, formed a normal cylindrical root with an apical meristem. The small wedge-shaped fragments, *B*, just back of the tip elongated from their original lengths of 0.5 and 0.8 mm. to 5 and 8 mm. respectively. The fragments, *C*, elongated from 1 and 1.5 mm. to 6 and 8 mm. respectively and formed numerous branch roots. The cells in *C* were large, 150 μ by 105 μ as compared with normal cells of 42 μ by 18 μ. It is particularly interesting that normal polarity is shown by the wedge shaped pieces. Although they did not develop into normal roots, the cells of which they were originally composed elongated in the same direction as they would have in the normal root, producing a filament and not an unorganized mass.

A pea root tip 2.5 mm. long was fragmented as shown in figure 15. The tip fragments, approximately 0.5 mm. long, formed a mound of cells. *B* and *C* each developed into normal roots measuring at the end of one week 6 and 4 mm. respectively.

One root tip of pea originally 2.4 mm. in length was fragmented as shown in figure 16. The wedge shaped piece, *B*, which included the major portion of the apical meristem, formed a complete root tip. The other two pieces, *A* and *C*, did not.

Discussion

In general the results of these experiments confirm the experiments of PRANTL (**6**), SIMON (**11**), LOPRIORE (**2, 3**), and NEMEC (**5**) on the regeneration of decapitated or split roots. In their experiments the decapitated or split roots were attached to the grain while in the experiments described in this paper excised root tips cultivated in pure culture were used.

Although accurate comparison of the results secured with excised and with attached root tips cannot be made, it would appear that the regenerative power of decapitated excised corn root tips under the conditions of our experiments is less than that of roots attached to the grain. Regeneration of a new root tip by the excised corn roots was observed only when decapitation was near the apex of the root tip. None of the basal pieces from fragments formed by cuts farther back in the meristem regenerated, although in SIMON'S experiments with attached roots they did. Excised wheat roots appear to have greater powers of regeneration than do those of corn, as re-

generation was observed in the majority of cases in which the excised root tip was decapitated back in the meristem some distance from the apex. The experiments with peas were too few to permit any conclusion other than that regeneration may occur.

SIMON, by anatomical studies and by experiments in which specific tissues of the root were removed and destroyed, concluded that the pericycle is primarily concerned in regeneration by decapitated roots. NEMEC, who studied regeneration in roots wounded in the meristem by cuts of various depths and directions, concluded that the interruption by the cut of "correlations" between the apex of the meristem and the remainder of the root was the chief cause of regeneration. The pericycle appeared to be the tissue through which the correlation was effective, as regeneration did not occur unless half or more than half of the pericycle was severed in wounding.

In our experiments the origin of regeneration from the plerome in decapitated excised wheat root tips and the failure of strips of the periblem to regenerate an apical meristem are in accord with the view that the pericycle is of prime importance in the regeneration of a new apical meristem and root cap by decapitated roots.

The results secured with excised root tips split longitudinally are similar to those found by SIMON and LOPRIORE for split roots attached to the plant. Evidently a quantitative relation is involved here. One quarter of an excised corn root tip divided longitudinally did not regenerate a new root tip, although a piece amounting to between one quarter and one half of the root tip did. This is of interest since NEMEC found that regeneration did not occur in the attached roots he used unless more than one half of the pericycle was severed.

These experiments, however, are exploratory rather than definitive. They show that a decapitated root tip 1 mm. or so in length contains within itself all the essentials for regenerating a new apical meristem and root cap if it is grown on an agar medium containing mineral salts and glucose. They suggest that the cultivation of excised root tips may permit a more complete analysis of the physiology of regeneration than is possible when the root tips are attached to the plant and supplied with all the materials which may be derived therefrom.

In addition to the observations made on the regeneration of decapitated excised root tips, the writers' experiments include some on the development of fragments of the apical meristem. Four types of development of isolated bits of the apex of a root tip were observed: (a) little or no development; (b) formation of filaments of parenchyma cells; (c) formation of a root in which stelar tissue differentiated but no apical meristem persisted; (d) formation of a normal root. The experiments do not permit such desirable analysis of these different types of development as, for example, to determine how far they depend upon the kind of tissue included, upon the quantity of tissue included, and upon the treatment (injury from cutting, nutrient conditions) of the isolated fragment. They suggest that if normal development is to occur in the fragment there must be included a portion of the plerome at the apex of the meristem between 0.1 and 0.2 mm. in length and equivalent to between one quarter and one half of that found at the root apex. Fragments of the root tip apex which do not contain this amount of the apical plerome fail to develop normally. The initial cells at the apex of the meristem need not be included in the pieces used. Here again the experiments are largely exploratory. Sufficient data have been secured, however, to show that the methods used may permit a more complete analysis of the factors involved in the existence of meristematic tissue in root tips and its differentiation.

Summary

1. Excised root tips of corn cultivated under sterile conditions on a nutrient medium grew in the majority of cases if they were 0.5 mm. or more in length, and in all cases if they were 0.9 mm. or more in length.

2. When excised root tips of corn and of pea 1–2 mm. long were divided longitudinally into equal parts, both pieces developed into complete roots on the nutrient medium used.

3. When excised root tips of corn and of pea 1–2 mm. long were divided longitudinally so that one part contained most of the plerome, the larger part developed on the media used into complete roots; the smaller grew but lacked one or more parts of a complete root.

4. When excised root tips of corn and of pea 1–2 mm. long were divided longitudinally into three or more pieces, a piece comprising less than one half of the root developed into a complete root; but one quarter, under the conditions of the experiment, did not.

5. When excised root tips of corn 1–2 mm. long were cut obliquely through the apex of the meristem, both pieces in some cases developed into complete roots; in others the terminal piece formed a filament of cells and the basal piece differentiated but possessed no apical meristem at the termination of the experiment.

6. When excised root tips of corn 1–2 mm. long were cut obliquely through the basal portion of the meristem, the tip fragment developed into a complete root but the basal fragment differentiated and formed no apical meristem.

7. When excised root tips of wheat 1–2 mm. long were cut longitudinally, neither piece developed into a complete root; both lacked an apical meristem at the completion of the experiment.

8. When excised root tips of wheat 1–2 mm. long were cut obliquely or transversely through the apex of the meristem, neither piece formed a complete root. The tip fragment formed masses of root cap cells and filaments of parenchyma cells; the basal portions elongated and differentiated but formed no apical meristem.

9. When excised root tips of wheat were cut obliquely or transversely through the basal portion of the apical meristem, the tip fragments developed into complete roots and the basal fragments regenerated a new apical meristem.

10. Four types of response of fragments of the apical meristem of root tips were observed as follows: (a) Little or no development; (b) formation of filaments of parenchyma cells; (c) formation of a root in which stelar tissue differentiated but no apical meristem remained; (d) formation of a normal root.

11. Injury from the cut may affect the character of the development of fragments of the apical meristem of roots.

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ADDITIONS TO THE GENUS EUPHORBIA L. AND TO CERTAIN GENERA OF THE COMPOSITAE

EARL EDWARD SHERFF

Euphorbia olowaluana sp. nov.—Frutex forsitan arborescens, ramosus; ramis brunneo-griseis (ultimis herbaceis saepius tenuissimis moniliformibus glabratis vel minutissime hispidulis, nodulis perspicuis). Folia disticha saepius squarrosa; petiolo tenui aegre puberulo 1–2 mm. longo; lamina oblonge lineari, apice obtusa vel acuta, basi inaequilaterali (sed tantum circ. 1–2 mm. lata), membranacea, glabrata, subtus venis obliquis brunneo-purpureis ornata, 1.5–2.5 (raro –3) cm. longa et 4–8 mm. lata; 2 stipulis in corpus interpetiolare primum triangulatum coalitis. Capitula plerumque solitaria in axillis etiam ad ramulorum apices. Involucrum subsessile campanulatum urceolatumve extus glabratum vel summam versus tomentellum, circ. 2.3 mm. altum, glandulis 4 transverse oblongis subcontiguis. Capsula cernua, profunde trigona, glabra, brunnea, circ. 2.3 mm. alta, coccis aegre carinatis carina atriore; stylis basi connatis, breviter bifurcatis ramis valde incrassatis; stipite pubescenti manifesto. Semina ovata, tetragona, brunnea (primum raro subgrisea), scrobiculata, 1.3–1.5 mm. longa.

Specimens examined: *Charles N. Forbes* 2242*M*, Olowalu Valley, Isl. Maui, May 7, 1920 (Herb. Field Mus.); *idem* 2341*M*, central ridge of Olowalu Valley, Isl. Maui, May 12, 1920 (type, Herb. Bishop Mus.).

Euphorbia olowaluana and its var. *gracilis* (*vide infra*) appear to constitute a species intermediate between *E. multiformis* H. & A. (as to often acutish leaves) and its var. *microphylla* Boiss. (as to herbaceous moniliform ultimate branchlets) on the one hand and its var. *manoana* and *E. celastroides* vars. *amplectens* and *mauiensis* (as to appearance of leaves, especially of the brownish purple venation) on the other.

In view of the existence of the many varieties of *E. multiformis* and

E. celastroides, the recognition here of *E. olowaluana* and the var. *gracilis* as apart from them must needs be somewhat arbitrary.

EUPHORBIA OLOWALUANA var. **gracilis** (Rock) comb. nov.; *E. lorifolia* var. *gracilis* Rock Indig. Trees Haw. Isls. 259, pl. 100. 1913.

EUPHORBIA HILLEBRANDII **palikeana** var. nov. Degener & Sherff.—Inflorescentia 3–10-cephala valde contracta 0.5–1.5 cm. longa nodulis bracteolatis numerosis perspicuisque.

Specimens examined: *Otto Degener, Kwan Park*, and *Will Bush* 8049, open woods, in third small valley northeast of Palikea, Isl. Oahu, Sept. 19, 1932 (type, Herb. N.Y. Bot. Gard.: cotype, Herb. Degener).

MR. OTTO DEGENER, the well-known authority on the flora of the Hawaiian Islands, courteously permits the use of his name, although somewhat provisionally, in joint authorship for this and certain other novelties (*vide infra*) collected by himself and his associates and described in this paper. He himself had been inclined to regard the indigenous Hawaiian Island species as deserving generic segregation from *Euphorbia*, a view which my own studies do not permit me to share.

EUPHORBIA HILLEBRANDII **wainianana** var. nov.—Rami adscendentes, virgati, sulculati, glabri, moderate nodosi, internodiis saepius 1.3–3 cm. longis. Foliorum petioli tenues patenti-hispiduli, 1–2 mm. longi; laminae elliptico-lanceolatae vel (saepe subrhomboide) oblanceolato-oblongae, apice subacutae vel acutae et fere mucronatae, basi inaequilaterali angustatae vix subtruncatae, glabrae, membranaceae, margine vix subrevolutae, infra paulo pallidiores, 2–4 cm. longae et 7–15 mm. latae; corporibus interpetiolaribus lato-triangularibus ∓0.5 mm. altis. Capitula axillaria terminaliaque (interdum ad ramulos minutos subcapilliformes axillares), solitaria vel subsolitaria. Involucrum campanulatum, minutum (sub 1.5 mm. altum), extus glabratum; lobis hirtis; glandulis 5, transverse oblongis, plus minusve contiguis, exappendiculatis; floribus masculis exsertis; pedicella glabra sub 1 mm. longa. Capsula submatura glabra, vix 2 mm. alta, coccis moderate carinatis, non sulcatis; stipite glabro, ∓4 mm. longo; stylis distinctis, fere usque ad medium bifurcatis, seminibus non visis.

Specimens examined: *Charles N. Forbes* (with *Dean Lake*) 1978*O*, Wainiana Ridge, Isl. Oahu, Oct. 27–30, 1914 (type, Herb. Bishop Mus.).

Euphorbia halemanui sp. nov.—Glabra, verisimiliter frutex 1–2 m. altus, ramorum internodiis plerumque 0.5–1.5 cm. longis. Folia opposita, tenuiter petiolata petiolis 5–10 mm. longis; lamina elliptice vel interdum subrhomboide oblonga vel obovata, apice obtusa nec emarginata, basi angustata sed vix inaeqilaterali, marginibus non vel parce subrevoluta, penninervi nervis divaricatis supra saepe obscuris, 4.5–8 cm. longa et 2–3.3 cm. lata; corpore stipulari interpetiolari, triangulari, scarioso, margine superiore fimbriato, 1–2 mm. alto. Inflorescentia contracta subglobosa (tantum 4–8 mm. alta), axillaris terminalisque, circ. 4–10 capitulis ad utrumque nodum. Involucrum urceolatum, extus glabrum, sub 2 mm. altum; glandulis 4 vel 5, transverse oblongis, exappendiculatis; pedicella minuta, glabra, sub 3 mm. longa; floribus masculis numerosis exsertis. Capsula submatura cernua stipite glabro sub 3 mm. longo, glaberrima, trigona, 2.2 mm. alta, coccis subacriter carinatis; stylis usque ad basim valde distinctis, lobis brevibus incrassatis.

Specimens examined: *Charles N. Forbes* 943*K*, Halemanu, Isl. Kauai, Jul. 3–Aug. 18, 1917 (Herb. Bishop Mus.); *idem* 1095(*a*)*K*, same locality and date (type, Herb. Bishop Mus.); *Dr. Carl Skottsberg* 1002, forest near Kokee Ranger Station, Isl. Kauai, Oct. 28, 1922 (Herb. Bishop Mus.).

Noticeable for its minute subglobose very much contracted clusters of heads, a cluster usually measuring 4–7 mm. tall.

Euphorbia forbesii sp. nov.—Glabra, probabiliter frutex plus minusve erectus; caulis internodiis saepius 1.5–3.5 cm. longis, siccis brunneo-rubris. Folia opposita, non numerosa, vix petiolata petiolis tantum circ. 1–3 mm. longis; lamina oblonge ellipticeve oblanceolata, apice obtusa et saepe paulo emarginata, basi inaequilaterali (unico latere saepe subcordata), crassiuscula vel membranacea, leviter revoluta, infra pallidiore, penninervi nervis lateralibus numerosis obscuris divaricatis, foliorum principalium 10–16.5 cm. longa et 2.5–4 cm. lata: stipulis interpetiolaribus late triangularibus superne angustatis omnino circ. 2 mm. altis. Inflorescentiae cymae axillares terminalesque, apertae, plus minusve valde decompositae ramis

ramulisque non valde patentibus, usque ad 7.5 cm. longae, 8–15-cephalae. Involucrum hemisphaericum, extus plus minusve pubescens, 1.5–3.5 mm. altum; lobis ovatis, hispidis; glandulis 4 vel saepius 5, latissime stipitatis, transverse oblongis (perspicue reniformibus), exappendiculatis, siccis purpureo-nigris; pedicella tenui, angulata, sulcata, glabrata vel saepius minute patenti-hispidula, saepius 1–2.2 cm. longa. Capsula submatura angulato-cylindrica, utrinque truncata, glabra, circ. 6 mm. alta, cernua, stipite glabrato circ. 3–4 mm. longo; stylis distinctis, ramis non valde separatis; seminibus non visis.

Specimens examined: *Charles N. Forbes* 1770*O*, Makuleia, slopes of Puu Kaala, Isl. Oahu, Apr. 26–May 16, 1912 (Herb. Bishop Mus.; Herb. Field Mus.); *idem* 2218*O*, Wahiawa ditch trail, Isl. Oahu, Aug. 17–20, 1915 (type, Herb. Field Mus.: cotypes, Herb. Kew; Herb. Missouri Bot. Gard.); *Joseph F. Rock* 3037, Kaukonahua, Wahiawa, Isl. Oahu, May 15, 1909 (Herb. Gray; Herb. N.Y. Bot. Gard.); *Harold St. John* 10629, shrubs 8 ft. tall, alt. 1800 ft., shady woods on ridge, South Opaeula Gulch, Paalaa, Koolau Mountains, Isl. Oahu, Nov. 9, 1930 (Herb. Berl.; Herb. Delessert; Herb. Kew).

Euphorbia clusiaefolia var. *grandifolia* Hillebr. (Fl. Haw. Isls. 395. 1888) was described by HILLEBRAND evidently from the lone sterile branch preserved in his herbarium (Herb. Berl.; the specimen is now before me). This branch came from "Makaleha in the Kaala range" and doubtless belongs to *E. forbesii* proper or else to its closest relative, *E. rockii* Forbes. Both of these species are found near Puu Kaala.

Euphorbia degeneri nom. nov.; *Euphorbia cordata* Meyen Beiträge Bot. Reise Erde 2:150. 1843 (*non* Räuschel *nec* Schrank).—Named for MR. OTTO DEGENER, who independently had noted (*in herb.*) the desirability of a new name for this species to avoid confusion with *E. cordata* Schrank.

EUPHORBIA DEGENERI **molokaiensis** var. nov.—Folia capsulaeque velutino-pilosae.

Specimens examined: *Otto Degener* 8067 *pro parte*, along beach in arid region, near Waiakanapo, Isl. Molokai, Apr. 19, 1928 (Herb. Degener; Herb. Field Mus.); *Joseph F. Rock* 7078 *pro parte*, Moomomi, Isl. Molokai, March, 1910 (type, Herb. Gray: cotype fragment, Herb. Field Mus.).

EUPHORBIA REMYI **waimeana** var. nov.—Folia moderate magis coriacea revolutaque, saepius obovato-oblonga, basi saepius subangustata et inaequilateralia, lamina plerumque circ. 7–8.5 cm. longa et 4–5 cm. lata. Unicum caput sine fructu visum.

Specimens examined: *Charles N. Forbes* 1043 *K*, Kalalau-pali, Waimea Drainage Basin, west side, Isl. Kauai, Jul. 3–Aug. 18, 1917 (type, Herb. Field Mus.: cotypes, Herb. Berl.; Herb. Delessert; Herb. Kew).

Confusion has arisen in literature owing to HORACE MANN'S publishing in 1867 (Proc. Amer. Acad. 7:201; Enum. Haw. Pl. no. 438) what purported to be an original description of *E. remyi* A. Gray, with Kauai cited first (*i.e.*, before Oahu) for the habitat. We must note, however, that some five years previously BOISSIER (*in* DC. Prodr. **15**[II]:1262. 1862) had published *E. remyi* A. Gray and cited Oahu first. In fact, BOISSIER listed two varieties, α and β, and based the var. α directly upon *Remy* 598 from Oahu.

I have found no other specimens matching the *Remy* type (now before me), and it may well be that the species proper is one of those probably numerous endemic Hawaiian species which have been exterminated within the past century. There are, however, various specimens from Kauai that fall into eleven more or less easily distinguishable varieties of *E. remyi*, one of these being the var. *waimeana*. This variety may be the first of the two forms described by HILLEBRAND (Fl. Haw. Isls. 395. 1888) as having been collected by *Knudsen* in Waimea. HILLEBRAND described the stipules as low, triangular, and 1–1.5 lines long. This holds for the very few stipules still observable toward tips of branches on my type, but the stipules lower down are represented merely by the whitish interpetiolar callosities which are conspicuous in this species.

EUPHORBIA REMYI **kauaiensis** Degener & Sherff var. nov.—Foliorum petioli tenues, 5–18 mm. longi; lamina elliptico-oblonga vel rarius -lanceolata vel subrhomboideo-oblonga, apice subacuta rarissime emarginata, basi plus minusve inaequilaterali cuneato-angustata vel raro subrotundata, subnitida, membranacea, 8–15.5 cm. longa et 3–5 cm. lata, penninervi nervis lateralibus divaricatis tenuibus manifestis, integerrima, non vel minutissime irregulariterque revoluta. Inflorescentia axillaris terminalisque, irregulariter

ramosa nodulis stipuliferis saepe 2–gemmatis, 1.5–4 cm. longa, plerumque 3–7-cephala. Involucrum siccum atrum vel purpureo-atrum, hemisphaericum, extus glabratum vel supra hispidulum, 3 mm. altum; lobis parvis, hirtis; glandulis plerumque 5, transverse oblongis, exappendiculatis; pedicella tenui, atra, glabrata vel sparsim setosa (saepe irregulariter minutissimeque plus minusve tenuipapillata), saepius 7–10 mm. longa. Capitula cylindrico-ovata, subacriter trigona, glabra, breviter stipitata (stipite semper recto vel subrecto videtur), 3.5–4 mm. alta; stylis usque ad basim distinctis, lobis brevibus vel brevissimis incrassatis. Semina castanea, acriter tetragona, basi truncata apice obtusa, faciebus rugoso-scrobiculata, circ. 2–2.2 mm. longa.

Specimens examined: *Otto Degener* 8093, near Hanapepe Falls, Isl. Kauai, June 19, 1926 (type, Herb. N.Y. Bot. Gard.: cotypes, Herb. Degener, two sheets; Herb. Field Mus.).

EUPHORBIA REMYI **pteropoda** var. nov.—Folia robuste petiolata petiolis latis marginatis 6–12 mm. longis; lamina late elliptico-oblonga, interdum minute emarginata, moderate revoluta, subcoriacea, 8–11 cm. longa et 4–4.8 cm. lata.

Specimens examined: *Abbé Urbain Faurie* 478, alt. 800 m., Kauhao, Isl. Kauai, February, 1910 (type, Herb. Delessert).

A variety quite distinct from the species proper and the other varieties in its robust, margined leaf petioles and wide leaf rachises. Only in the var. *olokelensis* (*vide infra*) do the leaf petioles or rachises tend at all to simulate those of var. *pteropoda* and then only occasionally and only to a slight extent.

EUPHORBIA REMYI **lydgatei** var. nov.—Folia tenuiter petiolata petiolis 7–10 mm. longis; lamina oblongo-obovata, apice ipso abrupte subacuta, basi angustata inaequilateralique, aegre vel parce revoluta, membranacea, 11–15.5 cm. longa et 5–7 cm. lata, nervis manifestis.

Specimens examined: *Reverend J. M. Lydgate*, Pole Line Trail, Wailua Mountains, Isl. Kauai (type, Herb. Bishop Mus.).

EUPHORBIA REMYI **leptopoda** var. nov.—Folia tenuiter petiolata petiolis obsolete hispidis 8–22 mm. longis; lamina saepius elliptico-oblonga (raro ovato-oblonga vel subrhomboideo-oblonga), apice obtuso interdum minute emarginata, basi rotundata vel subcordata

et saepius inaequilaterali, non vel tenuissime subrevoluta, nitida, saepius 9–13 cm. longa et 3–4.5 (–5.3) cm. lata.

Specimens examined: *Charles N. Forbes* 1095*K*, Halemanu, Isl. Kauai, Jul. 3–Aug. 18, 1917 (type, Herb. Bishop Mus.).

EUPHORBIA REMYI **kalihiana** var. nov.—Folia tenuiter petiolata petiolis 3–6 mm. longis; lamina subrhomboideo-oblonga, apice plerumque moderate acuminata, basi saepe inaequilaterali cuneato-rotundata, membranacea, leviter revoluta, tantum 4–7 (rarius –8.5) cm. longa et 2–3.5 cm. lata, venis supra obscuris.

Specimens examined: *Charles N. Forbes* 10*K*, at foot of Kalihi, Isl. Kauai, Jul. 8, 1909 (type, Herb. Field Mus., 3 sheets).

EUPHORBIA REMYI **wahiawana** var. nov.—Folia tenuiter petiolata petiolis 5–15 mm. longis; lamina nunc anguste nunc late oblongo-obovata, ad basim plus minusve inaequilateralem saepe sensim angustata, apice subacuminata, membranacea, leviter revoluta, 7–14 cm. longa et 3–5 cm. lata.

Specimens examined: *Charles N. Forbes* 177*K*, Wahiawa Mountains, Isl. Kauai, August, 1909 (type, Herb. Field Mus.); *Reverend J. M. Lydgate*, same locality (Herb. Bishop Mus.).

EUPHORBIA REMYI **olokelensis** Sk. & Sherff, var. nov.—Folia tenuiter vel sublate petiolata petiolis 4–12 mm. longis; lamina elliptico-oblonga vel subobovato-oblonga, basi saepe inaequilaterali nunc cuneato-angustata nunc subcordata, apice obtusa, membranacea, vix vel non revoluta, 6–10 cm. longa et 2–4.7 cm. lata. (*Cf.* C. SKOTTSBERG, Meddel. Göteborgs Bot. Trädgård **10**:121. 1936.)

Specimens examined: *Albert S. Hitchcock* 15220, alt. 1400 ft., Olokele Gulch, Isl. Kauai, Oct. 18, 1916 (Herb. U.S. Nat.); *Joseph F. Rock* 2078, Halemanu, Isl. Kauai, Feb. 14–26, 1909 (Herb. Bishop Mus.; Herb. Gray); *idem* 12936, Kaholuamanu, Isl. Kauai, October, 1916 (Herb. Bishop Mus.); *idem* 17108, same locality and date (Herb. Bishop Mus.); *Dr. Carl Skottsberg* 1050, innermost part of Olokele Valley, Isl. Kauai, Oct. 31, 1922 (type, Herb. Bishop Mus.).

Rock 17108, which, although coming from Kaholuamanu, is so close to the Olokele specimens that it is not varietally separable, has a mature capsule measuring 4 mm. long, the cocci obtuse on the back. The specimens from the type locality are without mature capsules.

The *Skottsberg* specimen had been labeled as new by its collector, DR. CARL SKOTTSBERG, Director of the Arboretum at Gothenburg, Sweden, and he very courteously permits the publication here of my description of it (this description being extended somewhat to include the other specimens cited).

EUPHORBIA REMYI **wilkesii** var. primum nom.; *Euphorbia remyi* var. *β*. Boiss. *in* DC. Prodr. **15**[II]: 1262. 1862.—Folia anguste oblonga, basi inaequilateraliter angustata, apice subacuta acutave raro vix subacuminata membranaceissima, non revoluta, petiolo tenui circ. 7–11 mm. longa, lamina 6–9 cm. longa et 1.5–2.6 cm. lata. Involucrum 2 mm. altum. Capsula tantum circ. 2.2 mm. alta, coccis acute carinatis haud sulcatis, seminis tetraquetri lateribus rugulosis; stipite valde reflexo.

Specimens examined: *United States Exploring Expedition*, Isl. Kauai, 1840 (type, Herb. U.S. Nat.).

ASA GRAY supplied a description of *Euphorbia remyi* to BOISSIER, as is shown in BOISSIER's citation "A. Gray in litt." and the additional note, "Descr. ex cl. A. Gray in litt." BOISSIER published the description in 1862 (*loc. cit.*). As already stated (see p. 584), BOISSIER listed two varieties, *α* and *β*, basing *α* on *Remy* 598, "absque fructu," from Oahu, and *β* on the material collected by the *United States Exploring Expedition under Captain Wilkes*, on Kauai. GRAY's description of the species *E. remyi* included the fruit. But since *Remy* 598 was "absque fructu" (and this is confirmed by an examination of GRAY's ample type specimen now before me), the fruit clearly must have been described from that in the packet of fragmentary but fruiting U.S. Explor. Exped. material which GRAY had before him and from which (as stated on the label) he sent "a little to Boissier."

Shortly after BOISSIER's work was published, HORACE MANN botanized in the Hawaiian Islands (May 4, 1864–May 18, 1865). He collected a specimen with larger, more acuminate leaves and capsules 4 (not about 2.2) mm. long, the cocci obtuse (not acutely carinate) upon their backs. ASA GRAY saw this material and his fragment of it, labeled "*Euphorbia Remyi* n. sp." in his own handwriting, is still preserved (in Herb. Gray). He evidently at once revised his supposedly unpublished description of *E. remyi* in an attempt to allow for the larger capsules and MANN, in 1867 (Proc. Amer. Acad.

7:202; Enum. Haw. Pl. no. 438), published the description as revised. Thus we read: "Capsules small, acutely triangular-3-lobed, and glabrous (immature?), or much larger, 2 lines long, the cocci obtuse on the back." The two types of fruit and even of leaves are varietally incongruous and I limit the var. *wilkesii* to plants typified by the original *Wilkes* (small-fruited) material.

EUPHORBIA REMYI **hanaleiensis** var. nov.—Folia membranacea, petiolo tenui ∓8 mm. longo; lamina late oblanceolato-oblonga, apice subacuminata, ∓12.5 cm. longa. Inflorescentia gracillima, ∓1.7 cm. longa, ramosa, ∓4-cephala, pedicellis tenuibus glabris 4–7 mm. longis; involucro ∓3 mm. lato, superne tomentello; coccis latera versus dense tomentosis, circ. 4 mm. longis; seminibus tetragonis, scrobiculatis, circ. 3 mm. longis.

Specimens examined: *Horace Mann* and *William T. Brigham*, Hanalei, Isl. Kauai, 1864–1865 (type, Herb. Cornell Univ.: cotype fragment, Herb. Gray).

Euphorbia skottsbergii sp. nov.—Frutex manifeste prostratus, ramosus, ramis tenuibus (ultimis tenuissimis) nodosis divaricatis novissimis tomentellis. Folia opposita, petiolo tenui tomentello 1–3 mm. longo; lamina diverse obovata oblonga oblongo-elliptica vel obovato-subrhomboidea, apice obtusa rotundatave, basi saepe inaequilaterali cuneato-rotundata, margine vix subrevoluto plana vel subrepanda (apicem versus rarissime obsoleto-denticulata), membranacea, supra glabrata, infra plus minusve hispidula, plerumque 1–2 cm. longa et 7–13 mm. lata; stipulis interpetiolaribus vix 0.5 mm. altis. Capitula plerumque secundum ramulos minutissimos axillares disposita. Involucrum (non vere pedicellatum) minutum campanulatum, glabratum vel moderate hispidulum, tantum circ. 1–1.1 mm. altum; lobis minutis, erecte oblongis, fimbriatis; glandulis 4 rarius 5, transverse oblongis, exappendiculatis, remotis vel fere contiguis; florum masculorum pedicellis exsertis. Capsula minuta glabrata, tantum circ. 1.6 mm. alta, cernua, breviter hispidula; coccis parce carinatis, non sulcatis; stylis distinctis, fere usque ad medium bifurcatis. Semina ovata, tetragona, basi truncata, apice obtusa, cinerea, papillato-scrobiculata, circ. 1.1 mm. longa.[1]

[1] *Cf.* C. SKOTTSBERG, Meddel. Göteborgs Bot. Trädgård 10:122. 1936.

Specimens examined: *Otto Degener* 8050, arid fossil reef, between Barbers Point and Pearl Harbor, Isl. Oahu, May 8, 1932 (Herb. Degener; Herb. Field Mus.; Herb. N.Y. Bot. Gard.); *Charles N. Forbes* 2330*O*, coral plain below Ewa and Sisal, Isl. Oahu, Mar. 14, 1916 (Herb. Field Mus., 4 sheets); *Dr. Carl Skottsberg* 122, Ewa coral plain, Isl. Oahu, Aug. 11, 1922 (type, Herb. Bishop Mus.); *O. H. Swezey*, Isl. Oahu (Herb. Bishop Mus.).

The name is given in honor of DR. CARL SKOTTSBERG (Director of the Arboretum of Gothenburg, Sweden), who kindly placed his entire collection of Hawaiian *Euphorbiae* at my disposal for study.

EUPHORBIA SKOTTSBERGII **kalaeloana** var. nov.—Erecta, ramosior, ramulis ultimis saepius capilliformibus. Folia minora, saepius 5–9 (raro –14) mm. longa.

Specimens examined: *Charles N. Forbes* (with *C. M. Cook*) 1760*O*, near Sisal, Isl. Oahu, Feb. 12, 1912 (Herb. Field Mus.); *Joseph F. Rock* 17034, coral plain under Algaroba, back of Barbers Point, Isl. Oahu, November, 1919 (1st type sheet, Herb. Gray; 2nd type sheet, Herb. Bishop Mus.).

EUPHORBIA SKOTTSBERGII **audens** var. nov.—Folia patentia vel patenti-reflexa, utroque margine plerumque 3–10-denticulata dentulis acribus induratis subpatenti-antrorsis. Semina vix longiora (circ. 1.3 mm. longa).

Specimens examined: *Charles N. Forbes* 620*Mo*, beach near Ka Lae Ka Ilio Ilio, Isl. Molokai, Mar. 25, 1915 (type, Herb. Bishop Mus.: cotype, Herb. Missouri Bot. Gard.).

EUPHORBIA SKOTTSBERGII **vaccinioides** var. nov.—Folia plerumque elliptica vel anguste obovato-elliptica.

Specimens examined: *Joseph F. Rock* 14072, west end flats, Isl. Molokai, April, 1918 (type, Herb. Bishop Mus.).

Euphorbia festiva sp. nov.—Frutex, unico ramo glabro sulcato sub 1.7 dm. longo viso, nodoso internodiis 1–1.4 cm. longis et 1–2 mm. crassis; ramulis suberectis tenerrimis (0.5–1 mm. crassis) plerumque 0.5–1 cm. longis, angulatis, sulculatis, superne sparsim hispidulis, nodosis nodis duplo crassioribus. Folia disticha, petiolo tenui glabrato tantum circ. 1 mm. longo; lamina oblonga vel rarius ovato-oblonga, apice saepe minutissimo-emarginato obtusa vel subro-

tundata, basi inaequilaterali eleganter lateque subcordata vel etiam moderate cordata, utroque margine angustissime indurata et non vere revoluta sed plerumque obsoletissime 1–8-dentulata, membranacea, utrinque glabra, infra pallidiore, 7–16 mm. longa et 5–11 mm. lata; stipulis interpetiolaribus triangularibus, pubescentibus, sub 1 mm. altis. Capitula terminalia (saltem pro 8 ramulis; nullis lateralibus repertis), solitaria. Involucrum subsessile, campanulatum, extus glabratum vel superne hispidulum, circ. 1.8–2.1 mm. altum; lobis hirtis; glandulis plerumque 5, transverse oblongis, non vel obsolete appendiculatis, plus minusve contiguis; floribus masculis exsertis. Capsula matura non visa; stylis basi connatis, valde bifurcatis ramis incrassatis.

Specimens examined: *Thomas Nuttall*, Isl. Oahu, 1835 (type, Herb. Kew). NUTTALL had designated the type as a new species (*Euphorbia pauciflora* Nutt. *ex* Seem. Fl. Vit. 1:216. 1865–68; *non* Duf. *nec* Hill), but later EDMOND BOISSIER determined it as *Euphorbia multiformis* H. & A. The oblong, basally wide-cordate or wide-subcordate leaves, with their obsolete yet definitely visible teeth, easily distinguish the *Nuttall* plant, however, from that species. Among the many specimens of Hawaiian *Euphorbiae* studied by me from the principal European and American herbaria, no others have been found to belong here, and it may well be that during the century since NUTTALL collected on Oahu the species has become extinct.

EUPHORBIA MULTIFORMIS var. **sparsiflora** (Heller) comb. nov.; *E. sparsiflora* Heller, Minnesota Bot. Studs. 1:846, pl. 51. 1897; *E. palustris* Heller, *loc. cit.* 847.

EUPHORBIA MULTIFORMIS **kaalana** var. nov.—Ramuli tenues sed perspicue nodosi, tomentelli, internodiis numerosis saepe tantum 3–6 mm. longis. Foliorum petioli tomentelli circ. 1–1.5 mm. longi; lamina plus minusve obovata, membranacea, basi inaequilaterali, apice rotundata saepe etiam retusa, subtus argenteo-tomentella, sub 2 cm. longa, venis obsoletissimis. Capitula numerosiora, saepe ad ramuli minimi axillaris apicem 2–5-congregata, perspicue tomentosa, circ. 3 mm. lata, glandulis brunneis plus minusve glabratis, antheris numerosissimis. Capsulae pilosulae, circ. 2.5 mm. altae et circ. 3 mm. latae. Semina subtetragona, scrobiculata, 1.5 mm. longa.

Specimens examined: *United States Exploring Expedition under Captain Wilkes*, "Kaala Mts., Wai-Nai," Isl. Oahu, 1840 (type, Herb. U.S. Nat.: cotype fragments, Herb. Bishop Mus.; Herb. Gray; Herb. Missouri Bot. Gard.).

A transitional form which might almost as well be considered a variety of *E. celastroides* Boiss. It was confused by ASA GRAY with *E. multiformis* var. *tomentella* Boiss., a plant with leaf blades mostly 2–3 cm. long, their veins conspicuous underneath, internodes of branchlets more elongate, capitula singly disposed, stamens fewer, etc.

EUPHORBIA MULTIFORMIS **haleakalana** var. nov.—Habitu varietati *microphyllae* Boiss. simillima. Rami foliorum petiolique hispidi. Involucrum extus nunc glabratum nunc (praecipue summam versus) patenti-hispidum; glandulis saepius patentibus; pedicella dense divaricateque albo-hispida. Capsula circ. 1.5 mm. alta, stipite patenti-hispido.

Specimens examined: *Charles N. Forbes* 2010*M*, Auwahi, south slope of Haleakala, Isl. Maui, Mar. 18, 1920 (type, Herb. Field Mus.).

EUPHORBIA MULTIFORMIS **kapuleiensis** Degener & Sherff, var. nov.—Habitu varietati *microphyllae* similis. Frutex circ. 1.8 m. altus, gracillimus, ramis elongatis subpendulisque, ramulis ultimis subcapilliformibus vel etiam capilliformibus; internodiis plerumque elongatioribus; foliis plerumque lineari-oblongis vel anguste ellipticis, petiolo 2–3.5 mm. longo, lamina sub 3 cm. longa.

Specimens examined: *Otto Degener* 8053, 6 ft. tall with long, almost drooping branches, rare, up ridge called Kapulei to east of white mountain Kaholoapele and back in east gully, Isl. Molokai, June 25, 1928 (type, Herb. Delessert: cotypes, Herb. Degener; Herb. Field Mus.; Herb. N.Y. Bot. Gard.); *idem* 8056, on arid cliffs, second eastern gulch, Wawaia, Isl. Molokai, June 27, 1928 (Herb. Degener, 3 sheets; Herb. Field Mus.); *idem* 8061, one of the dry valleys between Kamalo and Kaunakakai, Isl. Molokai, Jul. 29, 1928 (Herb. Degener, 2 sheets; Herb. Delessert; Herb. Field Mus., 2 sheets).

EUPHORBIA MULTIFORMIS **manoana** var. nov.; *E. multiformis* Gaud. (*saltem pro magna parte*), Bot. Freycin. Voy. 100. 1830 (*nom.*

subnudum); Boiss. *in* DC. Prodr. **15**[II]: 11. 1862 (*ex plantis Eschscholtzii, Maximoviczii, Macraeique, etiam descriptione et syn. Gaud.; excl. syn. Hook. & Arn.*).—Rami juniores glabri (pro typo) vel patenti-hispiduli. Folia saepius elliptico-oblonga vel cuneate oblanceolata, apice plerumque rotundata vel truncato-rotundata et saepe retusa, interdum facie inferiore hispidula et saepe petiolo tomentulosa. Involucrum extus basim versus papillato-hirtum sed summam versus dense tomentosum.

Specimens examined: *Otto Degener H*-213, on Tantalus side of Manoa Valley, Isl. Oahu, Feb. 12, 1923 (Herb. Degener, 2 sheets; Herb. Field Mus.; Herb. N.Y. Bot. Gard., 2 sheets); *Frederick Eschscholtz*, Isl. Oahu, 1816–1817 (Herb. Delessert; Herb. Gray; Herb. Kew); *Charles N. Forbes*, ridge west of Kalihi Valley, Isl. Oahu, Aug. 18, 1908 (Herb. Field, 2 sheets; Herb. Missouri Bot. Gard.); *idem* 358*H*, growing 12 ft. tall, with drooping branches, Kanehaha, Kona, Isl. Hawaii, Jul. 25, 1911 (Herb. Bishop Mus.); *idem* 1660*O*, Pacific Heights ridges, Isl. Oahu, March, 1911 (Herb. Field Mus., 2 sheets); *Charles Gaudichaud*, Hawaiian Isls., 1819 (Herb. Berlin); *Dr. William Hillebrand*, Honolulu, Isl. Oahu (Herb. Mus. Vienna); *Lay* and *Collie* (*Capt. Beechey's Voyage*), Isl. Oahu, 1826–1827 (Herb. Mus. Vienna); *James Macrae*, Isl. Oahu, May 20, 1825 (Herb. Berlin; Herb. N.Y. Bot. Gard.; Herb. Mus. Vienna); *Maximovicz* 145, Honolulu, Isl. Oahu (type, Herb. Kew: cotype, Herb. Berlin); *George C. Munro* 69, Maluea, Isl. Lanai, Dec. 27, 1913 (Herb. Bishop Mus.); *idem* 80, *eodem loco et tempore* (Herb. Bishop Mus., 2 sheets); *Joseph F. Rock*, right-hand branch of Wailupe Valley, Isl. Oahu, Apr. 14, 1918 (Herb. Bishop Mus.); *idem* 8126, Koele, Isl. Lanai, Aug. 3, 1910 (Herb. Field Mus.); *Carl Skottsberg* 1073, Nuuanu-Pauoa ridge, Isl. Oahu, Nov. 5, 1922 (Herb. Bishop Mus.); *Adelbert Von Chamisso* 182, Isl. Oahu, 1816–1817 (Herb. Berlin; Herb. Leningrad).

BOISSIER, to whom this variety represented the species proper, described the plants as glabrous. In two of the collections cited by him (*Eschscholtz's* and *Macrae's*), however, the younger branchlets and leaf petioles and lower surfaces are more or less hispidulous; so also for at least some of Gaudichaud's original material, of which I have seen KUNTH'S ample fragment (Herb. Berlin). The difference

in pubescence seems hardly constant enough to justify attempts at further than varietal delimitation.

EUPHORBIA CELASTROIDES var. **stokesii** (Forbes) comb. nov.; *E. stokesii* Forbes, Bishop Mus. Occas. Papers **5**: 108, pl. 1. 1913.

EUPHORBIA CELASTROIDES **moomomiana** var. nov.—Multum ramosa; ramis rubidis, crassis, conferte foliatis, internodiis plerumque 3–6 mm. crassis et duplo triplove longis. Folia perspicue disticha imbricataque, opposita, saepius reflexa; lamina subrigida, obovata, apice rotundata vel truncata interdum emarginata (raro etiam utroque latere apicem versus minute 1-sinuata), usque ad basim minute auriculato-cordatam sensim angustata, margine integra et subrevoluta, caeruleo-viride, infra pallidiore, 3–4.8 cm. longa et 1.8–2.8 cm. lata; corpore stipulari marginaliter fimbriato-hispido, 1.5–3.2 mm. alto. Capitula solitaria axillariaque vel 2–5 secundum axem incrassatum brevemque ex axilla ortum; pedicella glabra, ∓1 cm. longa. Capsula circ. 2 mm. alta et fere 3 mm. crassa.

Specimens examined: *Edward L. Caum* 10, Kaula, Hawaiian Isls., Aug. 17, 1932 (Herb. Bishop Mus.); *Otto Degener* 8069, forming 1×2 yard mass, in pure sand on leeward side of large dune, extremely dry region, Moomomi, Isl. Molokai, Apr. 25, 1928 (Herb. Degener, 2 sheets; Herb. Field Mus.; Herb. N.Y. Bot. Gard.); *George C. Munro* 494, Moomomi sand hills, Isl. Molokai, Jul. 26, 1922 (Herb. Bishop Mus.); *Joseph F. Rock* 14014, Moomomi, Isl. Molokai, April, 1918 (type, Herb. Bishop Mus., 3 sheets).

The *Munro* plant appears to have been regarded as new by ROCK, but at the Bishop Museum both the *Munro* and the *Rock* plants had been referred to the deceptively similar *Euphorbia stokesii* (my var. *stokesii*). The latter plant differs strongly, however, in having: rameal internodes much shorter and thicker; the much more numerous leaves so close that in the herbarium specimens they present a highly imbricated effect, their blade only very rarely with an indentation on each side of and a few millimeters from the midnerve's distal end; the tendency to bear thickened, abbreviated, axillary branches which are mere axes to hold the capitula; the shorter capsule, etc.

EUPHORBIA CELASTROIDES **kaenana** var. nov.—Frutex, ramis crassis et valde nodosis, cortice saepe albo-griseo et demum valde rugoso. Folia opposita, disticha, secundum ramulorum ultimorum brevissimorum 2–4 mm. crassorum partem tertiam (vel dimidiam) superiorem moderate conferta, inferiora saepe reflexa; petiolo gracili, glabro, 2–3 mm. longo; lamina late vel anguste oblongo-oblanceolata, apice obtusa vel truncato-subemarginata, inferne usque ad basim inaequilateralem truncato-subcordatam 3–5 mm. latam cuneate vel subcuneate angustata, pallide viridi, membranacea, plerumque 3–4.5 cm. longa et 1–1.6 cm. lata. Cymae multum contractae, axe ramisque crassiusculis nodosisque (bracteis stipularibus numerosis perspicuissimis); capitulis saepe 5–7, sessilibus vel subsessilibus; involucro extus glabrato vel minute setuloso nisi lobis hirsutis. Capsula subsessilis, glabrata, demum subnigra, circ. 2.5 mm. alta; seminibus extus subgriseis (subextus rubidis), biconvexo-tetragonis, scrobiculatis, apice baseque inaequilateralibus, tetragonis, 1.2–1.4 mm. longis.

Specimens examined: *Otto Degener, W. Hirai,* and *Kwan Kee Park* 8038, among rocks in arid region, near Kaena Point, Isl. Oahu, Mar. 21, 1931 (Herb. Degener; Herb. Field Mus.; Herb. N.Y. Bot. Gard.); *Charles N. Forbes* 1654*O*, between Makua Valley and Kaena Point, Isl. Oahu, Feb. 25, 1911 (Herb. Field Mus.); *idem* (*cum Dean Lake*) 2280*O*, talus slopes, Kaena Point, Isl. Oahu, Dec. 16, 1915 (Herb. Bishop Mus.); *Vaughan MacCaughey*, Kaena uplands, Isl. Oahu, Mar. 28, 1915 (type, Herb. Bishop Mus.).

EUPHORBIA CELASTROIDES **waikoluensis** var. nov.—Frutex, ramis subrubris moderate tenuibus. Folia moderate numerosa sed raro imbricata, pallida vel glaucescentia; petiolo subatro, minute puberulo, tantum circ. 1–2 mm. longo; lamina subrhombico-ovali, apice obtusa vel rotundata et interdum subemarginata, basi lata (4–8 mm.) et truncato-subcordata, plerumque 2.5–3.8 cm. longa et 1.5–2.5 cm. lata. Capitula (pauca visa) solitaria vel subsolitaria, axillaria vel terminalia; involucro praecipue summam versus pubescenti; pedicella brevi (1–3 mm.). Capsula (unica immatura visa) nigra.

Specimens examined: *Joseph F. Rock* 6191, on beach of Waikolu, Isl. Molokai (type, Herb. Field Mus.).

EUPHORBIA CELASTROIDES **haupuana** var. nov.—Sine dubio fruticosa; ramis ligneis, gracilibus, griseo-nigris, ultimis numerose

perspicueque nodosis (internodiis plerumque 3–7 mm. longis et 1–2.5 mm. crassis). Folia ut apud speciem ipsam (sed lamina minora et plerumque sub 3 cm. longa et 2 cm. lata apice plerumque obtusa rarissime retusa), ad ramorum apices subconferta. Capitula plerumque tantum 1 vel 2 ad unicum nodum; pedicella tenui, glabra, 4–7 mm. longa. Capsula (unica valde immatura visa) nigra.

Specimens examined: *Charles N. Forbes 20K pro parte*, near Lihue, Isl. Kauai, Jul. 9, 1909 (Herb. Field Mus.); *idem 600K*, Nonou Mountains, Isl. Kauai, Oct. 16–17, 1916 (Herb. Field Mus.); *Joseph F. Rock* 2444 "Haupu Lihue," Isl. Kauai, Mar. 18, 1909 (type, Herb. Bishop Mus.: cotype, Gray).

EUPHORBIA CELASTROIDES **kohalana** D. & S. var. nov.—Frutex, laxe ramosus; ramis subrubris, tenuibus, internodiis longis (saepe 2–4 cm.), nodis non perspicuis; ramis ultimis tenuissimis (∓1 mm. crassis), moderate foliatis et saepe ad apicem etiam ad 1 vel 2 nodos 1 vel 2 capitula brevissimi-pedicellata (pedicella 1–3 mm. longa) gerentibus. Folia angustius petiolata; lamina ovali vel obovata (raro paulum subrhomboidea), caeruleo-viridi vel glaucescenti, apice rotundata vel emarginata, basi plus minusve inaequilaterali anguste vel late rotundata vel truncato-subcordata, 2.5–4 cm. longa et 1.5–2.5 cm. lata.

Specimens examined: *Otto Degener* 8037, Kohala, Isl. Hawaii, Mar. 22, 1930 (type, Herb. Delessert: cotypes, Herb. Field Mus., 2 sheets; Herb. N.Y. Bot. Gard.); *Joseph F. Rock* 14032, without data (Herb. Bishop Mus.; a specimen without flowers or fruits and with the leaves mostly less blunt or rounded at apex).

EUPHORBIA CELASTROIDES **kealiana** var. nov.—Probabiliter fruticosa et mattae plus minusve similis; ramis subnigris, nodis numerosis perspicuisque, internodiis nunc 3–7 mm. nunc ∓1 cm. longis et ∓3 mm. (vel ramorum ultimorum 1–1.5 mm.) crassis. Folia ut apud speciem ipsam sed demum magis membranacea, lamina usque ad 5 cm. longa et 3.3 cm. lata, colore probabiliter diversa. Capitula non visa.

Specimens examined: *Abbé Urbain Faurie* 477, on rocks of the shore at Kealia, Isl. Kauai, January, 1910 (type, Herb. Brit. Mus.); *Charles N. Forbes 20K pro parte*, near Lihue, Isl. Kauai, Jul. 9, 1909 (Herb. Field Mus.).

EUPHORBIA CELASTROIDES **halawana** var. nov.—Frutex ramosissimus foliosissimus; ramis ultimis subultimisque subrubris longitudinaliter valde rugosis (saltem demum), internodiis plerumque 1–2 mm. crassis. Folia minus decidua, demum subbrunnea, multo minora; petiolo tenui, 1–2 (raro –3) mm. longo; lamina diverse ovali obovata subrhomboideo-obovata vel suborbiculari, apice rotundata vel truncato-emarginata, basi inaequilaterali et plerumque sublata rotundata vel truncato-subcordata, pro paucis foliis caulis usque ad 3.5 cm. longa et 2 cm. lata sed pro multitudine foliorum ramorum plerumque 1–2.5 cm. longa et 0.8–2 cm. lata (vel pro minutis saepe numerosis foliis capitula subtendentibus ∓4 mm. longa et ∓4 mm. lata). Capitula subsessilia, plerumque solitaria, saepe ad et prope summam ramuli lateralis pumili folioso-bracteolati gesta; involucro saltem supra extus tomentoso. Capsula glabra vel aegre pubescens, viridi-brunnea, sessilis vel subsessilis, ∓3 mm. alta; seminibus subgriseis, scrobiculatis, 2 mm. longis.

Specimens examined: *Otto Degener* 8054, dry coastal cliffs, west side of Halawa Valley, Isl. Molokai, June 20, 1928 (Herb. Degener, 3 sheets; Herb. Field Mus., 2 sheets); *idem et Ross S. Bean* 8041, Isl. Oahu, Apr. 12, 1931 (Herb. Degener); *Degener* and *Noel Krauss* 8036, sunny ridge, west slope of Kahana Valley, Isl. Oahu, Feb. 26, 1929 (Herb. Degener; Herb. Field Mus.; Herb. N.Y. Bot. Gard.; type of forma *kahanana, ramulis siccis minus rugosis costatisve, foliis numerosioribus, inflorescentiis saepius foliis minimis subtentis*); *Abbé Urbain Faurie* 472, on shore at Halawa, Isl. Molokai, June, 1909 (Herb. British Mus.); *Joseph F. Rock* 14041, near Halawa, Isl. Molokai, April, 1918 (type and cotype, Herb. Bishop Mus.).

EUPHORBIA CELASTROIDES **saxicola** Degener & Sherff, var. nov.—Habitu var. *halawanae* moderate similis. Folia plerumque paulo minora et saepius obovata, apice emarginata. Involucrum plerumque breviter (1–4 mm.) sed manifeste pedicellatum. Capsula (sessilis) demum brunneo-atra vel atra.

Specimens examined: *Otto Degener* 8088, along rocky shore, Kohala, Isl. Hawaii, Aug. 9, 1926 (type, Herb. Delessert; cotypes, Herb. Degener, 2 sheets; Herb. Field Mus.).

EUPHORBIA CELASTROIDES **humbertii** var. nov.—Probabiliter fruticosa; ramis adscendentibus, tenuibus, ultimis plerumque ad

internodia (saepe 3–9 mm. longa) 1–1.6 mm. crassis, nodis perspicuis. Folia moderate conferta secundum tertiam vel dimidiam superiorem ramulorum, petiolo tenui 2–4 mm. longo; lamina cuneate et saepe subfalcate oblanceolata, apice obtusa vel truncata vel rotundata sed raro retusa, basi inaequilaterali-truncata et tantum circ. 1.5–3 mm. lata, caerulea vel glaucescenti, submembranacea, plerumque 2–4 cm. longa et 0.7–1.5 cm. lata. Capitula multa sed plerumque tantum 1 vel 2 ad unum nodum, plerumque elongato-pedicellata; involucro extus glabrato; pedicella tenui, rigida, suberecta et demum ∓13 mm. longa. Capsula glabra, demum brunneo-atra, circ. 2.5 mm. alta.

Specimens examined: *Jules Remy* 595, Kauai, 1851–1855 (type, Herb. Gray: cotypes, Herb. Paris, 2 sheets).

Named for PROFESSOR H. HUMBERT of Paris, without whose invaluable assistance my treatments of this and several other members of the genus *Euphorbia* would have been greatly handicapped, if not indeed precluded.

EUPHORBIA CELASTROIDES **nematopoda** var. nov.—Habitu var. *humbertii* similis; foliis minus confertis, capitulis paucioribus et in pedicellis parte tertia vel dimidia longioribus etiam gracilioribus magis flexilibus non rigidis suberectisque.

Specimens examined: *Charles N. Forbes* 726*K*, at left-hand side of Kipu Kai Gap, Haupu Range, Isl. Kauai, Nov. 1, 1916 (type, Herb. Bishop Mus.).

EUPHORBIA CELASTROIDES var. **lorifolia** (A. Gray) comb. nov.; *E. multiformis* var. *lorifolia* A. Gray *ex* H. Mann, Proc. Amer. Acad. **7**:202 (Enum. Haw. Pl., no. 439, var. δ). 1867; *E. lorifolia* Hillebr. Fl. Haw. Isls. 395. 1888 (*ex syn. A. Grayi*); *E. rivularis* Heller, Minn. Bot. Studs. **1**:846, pl. 51. 1897.

EUPHORBIA CELASTROIDES **hanapepensis** var. nov.; *E. celastroides* Heller, Minn. Bot. Studs. **1**:844. 1897 (*non* Boiss.).—Arbor parva, laxe ramosa, caule brevi; ramis minoribus vel junioribus rigidis et internodiis brevibus (plerumque 5–12 mm.), subbrunneo-atris vel saltem demum subgriseo-atris. Folia ramulorum apices versus subconferta, petiolo tenui et 1–4 mm. longo; lamina lineari-oblanceolata vel late oblanceolata, apice rotundata vel subobtusa, basi angusta (plerumque sub 3 mm. lata), magis membranacea et supra subviridiore, infra moderate flavido-argentea, 2–5 cm. longa et 0.7–1.7 cm.

lata. Capitula multo numerosiora quam apud speciem ipsam, solitaria ad nodos vel saepius in cymis 2–5-cephalis et in ramulorum lateralium foliosorum brevium axillis disposita; involucro extus glabro; pedicella tenui, glabra, 3–8 mm. longo. Capsula glabra, demum atro-brunnea, seminibus subgriseis, scrobiculatis.

Specimens examined: *Abbé Urbain Faurie* 476, on rocks, Hanamaulu, Isl. Kauai, December, 1909 (Herb. Bishop Mus.; Herb. Delessert); *A. A. Heller* 2429, along the Hanapepe River, near the Falls, Isl. Kauai, June 24–26, 1895 (Herb. Field Mus., 2 sheets; Herb. Gray; Herb. N.Y. Bot. Gard.); *Heller* (*similiter*) 2429, on the Hanapepe and Wahiawa Watershed, Isl. Kauai, June 24, 1895 (Herb. Kew); *Heller* (*similiter*) 2429, along the Hanapepe River, near the Falls, Jul. 2–8, 1895 (type, Herb. Missouri Bot. Gard.: cotype, Herb. U.S. Nat.).

EUPHORBIA CELASTROIDES **niuensis** var. nov.—Varietati *lorifoliae* similis. Foliorum petioli tenues, glabrati, 2–3 mm. longi; lamina oblonga, basi inaequilaterali-truncata et 3–5 mm. lata, apice obtusa rotundatave, glaucescenti, subtenui, ∓2.5 cm. longa et ∓1.2 cm. lata. Capitula ∓11-adgregata in cymulis 1.5 cm. longis, cymulorum ramulis patentibus vel recurvatis, pedicellis tenuibus sed brevibus (1–4 mm. longis). Capsulae siccae atro-brunneae, glabrae, sessiles vel subsessiles, circ. 2 mm. altae. Semina extus subgrisea subextus rubida, tetragona, tantum circ. 1–1.1 mm. longa, tantum moderate vel etiam indistincte scrobiculata.

Specimens examined: *Dr. William Hillebrand*, Niu, Isl. Oahu (type, Herb. Berlin).

Perhaps now extinct. The type is a mere fragment but very distinctive. It is the specimen referred to by HILLEBRAND (Fl. Haw. Isls. 395. 1888) for the Oahu material of *Euphorbia celastroides*. I have found no other material to match it.

EUPHORBIA CELASTROIDES **amplectens** var. nov.; *E. lorifolia* Hillebr. Fl. Haw. Isls. 395. 1888 (*pro parte parva et exclud. syn.* A. Gray *et* DC.; *cf. var. mauiensem*).—Nunc frutex prostratus erectusve nunc arborescens, ramosissima; ramis ultimis penultimisque griseo-atris, puberulis, non valde rugosis, nodis numerosis perspicuis, internodiis plerumque 1–2 mm. crassis. Folia polymorpha sed plerumque petiolo angusta pubescentia 1–3 mm. longa; lamina plerumque ellip-

tico-ovata vel anguste oblanceolata, apice truncata (etiam emarginata) vel rotundata vel obtusa, basi rotundata vel angustata, viridi vel demum plus minusve brunnescenti, infra interdum (saltem petiolum versus) puberula, plerumque, 1.5–3 cm. sed interdum usque ad 6 cm. longa. Capitula plerumque solitaria, subsessilia; involucro extus basim versus glabro vel glabrato summam versus saepe pubescenti vel perspicue tomentoso. Capsula glabra vel moderate puberula, sessilis vel subsessilis, demum (sicca) brunneo-viridis vel atro-brunnea, circ. 3 mm. alta. Semina nunc manifeste tetragona nunc obcompressa et (basi truncata tetragonaque excepta) 2-marginata, apice obtusa, extus grisea subextus rubida, scrobiculata, 1.7–2 mm. longa.

Specimens examined: *Anon.* 375, shrub about 4–6 ft. tall, Pololu Gulch, Kohala, Isl. Hawaii (Herb. Field Mus.); *Otto Degener* 8043, windswept ridge at beginning of forest, east ridge of Niu Valley, Isl. Oahu, Apr. 20, 1931 (Herb. Degener; Herb. Field Mus.; Herb. N.Y. Bot. Gard.); *idem* 8055, growing 3.5–5 ft. tall, with *Santalum*, Mokomoko Gulch, Isl. Molokai, June 7, 1928 (Herb. Degener); *idem* 8057, shrub 3 ft. tall, with *Lipochaeta degeneri*, in hot boulder-strewn region not far from ocean, near Kamakaipo, Isl. Molokai, May 16, 1928 (Herb. Degener); *idem* 8058, on moderately dry rocky slope, growing with *Exocarpus*, Kahuaawi Gulch, Isl. Molokai, May 12, 1928 (Herb. Degener; Herb. Field Mus.; Herb. N.Y. Bot. Gard.); *idem* 8060, East Ohia ridge, Isl. Molokai, Jul. 17, 1928 (Herb. Degener; Herb. Field Mus.; Herb. N.Y. Bot. Gard.); *idem* 8062, one of the dry valleys between Kamalo and Kaunakakai, Isl. Molokai, Jul. 29, 1928 (Herb. Degener; Herb. Field Mus.; Herb. N.Y. Bot. Gard.); *idem* 8076, dry rocky region, Kaupo Gap, Haleakala, Isl. Maui, Aug. 20, 1927 (Herb. Degener; Herb. N.Y. Bot. Gard.); *idem* 8077, northwest of Mt. Eke, Isl. Maui, Aug. 31, 1927 (Herb. Degener, 2 sheets; Herb. Field Mus.; Herb. N.Y. Bot. Gard.); *idem* 8078, arid rocky region, Ulupalakua, Isl. Maui, June 23, 1927 (Herb. Degener, 2 sheets; Herb. Field Mus.; Herb. N.Y. Bot. Gard.); *idem* 8087, arid *aa* lava desert, Hoopuloa, Isl. Hawaii, Aug. 26, 1926 (Herb. Degener, 3 sheets; Herb. Field Mus.); *idem* 8089, in tapestry forest, Kohala ditch trail, Kohala, Isl. Hawaii, Aug. 10, 1926 (Herb. Degener, 3 sheets; Herb. Field Mus.); *idem et Kwan Kee Park* 8045, in arid

rocky sunny region, second ridge east of Kuliouou Valley, near summit (ridge on west side of Kamehameha Farm School), Isl. Oahu, Oct. 25, 1931 (Herb. Degener, 2 sheets; Herb. Field Mus.); *David Douglas* 13, Hawaiian Isls., 1834 (Herb. Kew, 2 sheets); *Frederick Eschscholtz*, Isl. Oahu, 1816–1817 (Herb. Kew; *forma foliis E. multiformi valde adpropinquans*); *Abbé Urbain Faurie* 471, on rocks, Yao Valley, Isl. Maui, August, 1909 (Herb. Brit. Mus.); *Charles N. Forbes* 17*Mo*, Isl. Molokai, June, 1912 (Herb. Bishop Mus.); *idem* 43*L*, mountains near Koele, Isl. Lanai, June, 1913 (Herb. Field Mus., 2 sheets); *idem* 93*M*, Iao Valley, West Maui, June, 1910 (Herb. Field Mus.); *idem* 314*L*, Isl. Lanai, September, 1917 (Herb. Field Mus.); *idem* 355*Mo*, Kaluoohu Valley, Isl. Molokai, August, 1912 (type, Herb. Field Mus.: cotypes, Herb. Field Mus.; Herb. Missouri Bot. Gard.); *idem* 358*H*, Kanehaha, Kona, Isl. Hawaii, Jul. 25, 1911 (Herb. Bishop Mus.); *idem* 480*M*, Honakahau Drainage Basin, Isl. Maui, Sept. 25–Oct. 17, 1917 (Herb. Field Mus.); *idem* 533*Mo*, Halawa, Isl. Molokai, September, 1912 (Herb. Field Mus.); *idem* 1511*O*, Koko Head, Isl. Oahu, June 11, 1909 (Herb. Bishop Mus.); *idem* 1666*O*, Puu-O-Kona, Isl. Oahu, Mar. 14, 1911 (Herb. Field Mus.); *idem* 1775*M*, Isl. Maui, Dec. 9, 1919 (Herb. Bishop Mus.); *idem* 1913*M*, Nuu, south slope of Haleakala, Isl. Maui, Mar. 9, 1920 (Herb. Field Mus.); *idem* 2386*M*, Olowalu Valley, Isl. Maui, May 16, 1920 (Herb. Field Mus.); *idem* 2529*O*, Wailupe, Isl. Oahu, January, 1919 (Herb. Bishop Mus.); *Charles Gaudichaud*, Hawaiian Isls. (Herb. Missouri Bot. Gard., fragment *ex Herb. Delessertii*); *Dr. William Hillebrand*, Isl. Molokai, June 21 (Herb. Berlin; labeled *Euphorbia lorifolia* by HILLEBRAND); *idem* 48, Isl. Hawaii (Herb. Kew); *Albert S. Hitchcock* 14484, shrub or small tree on lava flow, Puuwaawaa, Isl. Hawaii, Aug. 30, 1916 (Herb. Bishop Mus.; Herb. U.S. Nat.); *James Macrae*, Isl. Maui, May, 1825 (Herb. Missouri Bot. Gard.; fragment *ex herb. Kunthii*); *idem*. Isl. Oahu, May 20, 1825 (Herb. N.Y. Bot. Gard.); *Horace Mann* and *William T. Brigham* 101 *pro parte*, Isl. Oahu, 1864–1865 (Herb. Field Mus.); *iidem* 427, ridge east of Nuuanu Valley, Isl. Oahu, 1864–1865 (Herb. Bishop Mus.); *Maximowitsch* 145, Honolulu, Isl. Oahu (Herb. Berlin; Herb. Kew); *George C. Munro* 45, outer forest, Kaiholena, Isl. Lanai, August, 1913 (Herb. Bishop Mus.); *Joseph F.*

Rock, Hawaiian Isls. (Herb. Field Mus., 2 sheets); *idem*, Honakahau Valley, Isl. Maui (Herb. Bishop Mus.); *idem* 8068, Hawaiian Isls. (Herb. Field Mus.); *idem* 8126, Koele, Isl. Lanai, Aug. 3, 1910 (Herb. Gray); *idem* 8359, Hawaiian Isls. (Herb. Field Mus.); *idem* 8677, East Maui (Herb. Field Mus.); *idem* 8679, *eodem loco* (Herb. Field Mus.); *idem* 17036, prostrate, near the ocean, Barbers Point, Isl. Oahu, November, 1919 (Herb. Bishop Mus., 2 sheets; Herb. Gray); *Dr. Carl Skottsberg* 1953, along road between Lind's and Pauwaawaa, Isl. Hawaii, Sept. 26, 1926 (Herb. Bishop Mus.); *John F. G. Stokes*, Isl. Molokai, 1909 (Herb. Bishop Mus.); *D. LeRoy Topping* (*Otto Degener* distrib. no.) 8075, Isl. Maui, Aug. 5, 1927 (Herb. Degener; Herb. Field Mus., 2 sheets); *Adelbert Von Chamisso*, Isl. Oahu, 1816–1817 (Herb. Missouri Bot. Gard., fragment *ex Herb. Berol.;* Herb. Petrop., 2 sheets); *Dr. Heinrich Wawra* 1852, Isl. Maui, 1868–1871 (Herb. Mus. Vienna, 2 sheets).

Similar to and apparently passing by many intergradations into the var. *mauiensis*, but with leaves tending to be more often obovate and less often linear, and with seeds commonly larger (1.7–2 mm. not 1.4–1.6 mm. long) and usually tetragonal (only rarely—for example, *Rock* 17036—obcompressed). Plants perhaps not averaging as tall as in var. *mauiensis*, but some of the specimens included here are from plants $\mp$3.6 m. tall (for example, *Forbes* 358*H*, "slender, 12 ft., drooping branches").—Apparently consists of several *formae* or races.

EUPHORBIA CELASTROIDES **mauiensis** var. nov.; *E. lorifolia* Hillebr. Fl. Haw. Isls. 395. 1888 (*pro parte magna et exclud. syn.* A. Gray *et* DC.; *cf. var. amplectentem*).—E frutice altitudinum minorum usque ad arborem parvam (3–6 m. altam et cum caule 1.5–2 dm. crasso) altitudinum majorum; ramis rigidis, nodosis, puberulis, internodiis brevibus. Folia breviter (1–2 mm.) petiolata, petiolo puberulo; lamina lineari vel lineari-oblonga vel rhomboideo-lineari, apice obtusa vel truncata et saepe retusa, basi paulum contracta etiam subtruncata inaequilateralique, glabra vel infra praecipue basim versus moderate puberula, 3–5 (rarius –7 vel etiam –9.5) cm. longa, demum (sicca) plus minusve brunneo-viridi, venis lateralibus ventraliter atris distinctisque. Capitula plerumque solitaria, sessilia subsessiliave; involucro extus pubescenti. Capsula puberula, sub-

sessilis, profunde 3-divisa, demum (sicca) brunneo-viridis vel atro-brunnea, ∓2.7 mm. alta. Semina nunc obcompresso-tetragona (2 marginibus interdum ad costas medianas reductis) nunc manifeste obcompressa et lateraliter tantum (nisi basi tetragona) 2-marginata, basi truncata et apice leviter rotundata, brunneo-grisea vel metallico-brunnea, scrobiculata, 1.4–1.6 mm. longa.

Specimens examined: *Anon.*, Isl. Maui (Herb. Bishop Mus., fragment *ex Herb. Berol.*); *Otto Degener* 8074, barren arid hill, *mauka* of McGregor, West Maui, Jul. 10, 1927 (Herb. Degener; Herb. N.Y. Bot. Gard.); *Charles N. Forbes*, 115*L* mountains near Koele, Isl. Lanai, June, 1913 (Herb. Bishop Mus.); *idem* 139*L*, *eodem loco et tempore* (Herb. Field Mus.); *idem* 223*L*, mountains at east end of Lanai, *eodem tempore* (Herb. Bishop Mus.; Herb. Field Mus.); *idem* 366*L*, Kaiholena, Isl. Lanai, September, 1917 (Herb. Field Mus., 2 sheets); *idem* 1104*M*, Kaupo Gap, Haleakala Crater, Isl. Maui, Aug. 10, 1919 (Herb. Field Mus.); *idem* 1811*M*, Kanaio, south slope of Haleakala, Isl. Maui, Mar. 2, 1920 (Herb. Bishop Mus.); *idem* 1812*M*, *eodem loco et tempore* (Herb. Field Mus.); *idem* 2048*M*, Auwahi, south slope of Haleakala, Isl. Maui, Mar. 20, 1920 (Herb. Field Mus.); *idem* 2091*M*, Auhi, south slope of Haleakala, Isl. Maui, Mar. 24, 1920 (Herb. Field Mus.); *idem et C. Montague Cook Jr.* 1*M*, Maunahooma, West Maui, May, 1910 (Herb. Bishop Mus.); *Dr. William Hillebrand* 45, small tree 15–20 ft. tall, Kula, East Maui, July, 1858 (Herb. Kew, 2 sheets); *idem et Rev. J. M. Lydgate*, Hawaiian Isls. (Herb. Bishop Mus.); *Albert S. Hitchcock* 14809, alt. 3000–5000 ft., Puu Kukui, West Maui, Sept. 24–26, 1916 (Herb. Bishop Mus.; Herb. U.S. Nat.); *Horace Mann* and *William T. Brigham* 389, sandy isthmus of Maui, 1864–1865 (type, Herb. U.S. Nat.: cotypes, Herb. Bishop Mus.; Herb. Field Mus.; Herb. Gray; Herb. Missouri Bot. Gard.); *George C. Munro* 66, Kaiholena, Isl. Lanai, August, 1913 (Herb. Bishop Mus.); *idem* 131, outer forest, ridge behind Kaiholena, Isl. Lanai, Sept. 28, 1913 (Herb. Bishop Mus.; Herb. Field Mus.); *Joseph F. Rock*, 8073, Mahana, Isl. Lanai, July, 1910 (Herb. Field Mus., 2 sheets; Herb. Gray; Herb. Mus. Vienna); *idem* 8560, gulches above Makawao, Isl. Maui, September, 1910 (Herb. Field Mus.; Herb. Gray; Herb. N.Y. Bot. Gard.); *Dr. Heinrich Wawra* 2343, Hawaiian Isls., 1868–1871 (Herb. Mus. Vien-

na); *idem* 2527, Isl. Maui, *eodem tempore* (Herb. Mus. Vienna); *Garrit P. Wilder*, Isl. Maui, 1913 (Herb. Bishop Mus.).

As stated under the foregoing variety (var. *amplectens*), that variety apparently passes into this. Because of the peculiarly obcompressed seeds, I had originally held this to be a valid species. The presence of forms of var. *amplectens*, however, in which forms most of the seeds are distinctly obcompressed (although still of greater length), seems to cast doubt upon the value of the obcompressed-seed character. Anyway, the local and visiting botanists who have collected the two forms have very commonly confused them under the single though erroneous designation, *Euphorbia lorifolia*.

EUPHORBIA ATROCOCCA **kokeeana** var. nov.—Folia pauciora, latiora (principalia plerumque 1.2–1.8 cm. lata), atro-viridia, venis lateralibus manifestis.

Specimens examined: *Otto Degener* 8094, Waimea Canyon near Kokee Camp, Isl. Kauai, June 30, 1926 (Herb. Degener, 2 sheets; Herb. Field Mus., 2 sheets; Herb. N.Y. Bot. Gard.); *Charles N. Forbes* 435*K*, Kaholuamanu, behind Waimea, Isl. Kauai, September, 1909 (Herb. Field Mus., 2 sheets); *Amos Arthur Heller* 2858, on Kaholuamanu, above Waimea, Isl. Kauai, Sept. 10–16, 1895 (Herb. Bishop Mus.); *idem* (*similiter*) 2858, *eodem loco*, Sept. 24–30, 1895 (Herb. Field Mus.); *idem* (*similiter*) 2858, *eodem loco*, Oct. 1–8, 1895 (Herb. Gray; Herb. Kew; Herb. Missouri Bot. Gard.; Herb. N.Y. Bot. Gard.; Herb. U.S. Nat.); *Joseph F. Rock*, Kaholuamanu, Isl. Kauai, October, 1911 (Herb. Field Mus.); *idem* 10099, below F. Gray's mountain house, Kaholuamanu, Isl. Kauai, October, 1911 (Herb. Bishop Mus.; Herb. Gray); *idem* 12933, Kaholuamanu, *eodem loco* (Herb. Bishop Mus.); *Dr. Carl Skottsberg* 1017, between Kokee and Mohihi, Isl. Kauai, Oct. 29, 1922 (type, Herb. Bishop Mus.).

On most specimens the apparently mature capsules are, as in the species proper, devoid of good seed. On two of the cited *Degener* specimens, however, a few ripe seeds were found. These were ovate-oblong in outline, truncate at base, obtuse at apex, acutely tetragonal with prominently carinate angles, reddish brown to brownish black, transversely scrobiculate, 1.8–2 mm. long.—Heller (Minnesota Bot. Studs. 1:844. 1897) described this variety as "a well

marked form, growing at an elevation of 4000 feet, near the edge of the woods."

EUPHORBIA ATROCOCCA **kilaueana** var. nov.—A specie ramis erectioribus elongatioribusque foliis tenuioribus eleganter spathulato-oblanceolatis apice saepius acutiusculis lamina 3–5.3 cm. longa differt. Inflorescentia ignota.

Specimens examined: *Abbé Urbain Faurie* 470, Kilauea, Isl. Kauai, January, 1910 (type, Herb. Brit. Mus.).

Coreocarpus johnstonii sp. nov.—Suffruticosa, e radice lignea erecta, ∓3 dm. alta, trichotome ramosissima ramis glabris vel parce hispidulis, lateralibus divaricatis et plerumque crassioribus, sicca non vel vix fragrans. Folia petiolo glabro vel interdum subhispidulo 3–8 mm. longo adjecto circ. 1–1.8 cm. longa, plerumque bipinnatisecta, segmentis ultimis crassiusculis lineari-oblongis vel subovatis subobtuse dentatis. Capitula numerosissima, cymose ad ramorum apices congregata, pansa ad anthesin ∓9 mm. lata et ∓6 mm. alta, pedicellis tenuibus vel tenuissimis minutissime 1–3-bracteolatis 1–4 cm. longis. Involucrum turbinatum, glabratum, bracteis paucis (plerumque 5 rarius usque ad 7), subaequalibus, ovatis, apice acutis, tantum circ. 3 mm. longis et quam disci floribus fere dimidio brevioribus. Flores ligulati circ. 3, albido-flavi; ligula oblongi, integri, +2.5 mm. longi. Achaenia exteriora corpore ipso 2.5–3 mm. longa et 1–1.3 mm. lata, alis plene in dentes nunc contiguos nunc separatos incisis, apice calvo; interiora angustiora, alarum dentibus saepe subremotioribus.

Specimens examined: *Ivan M. Johnston* 4293, common in dense low masses on a gravelly beach, San Pedro Bay, State of Sonora, Mexico, Jul. 7, 1921 (type, Herb. California Acad. Sciences).

I am indebted to MISS ALICE EASTWOOD, Curator of the Botanical Department, California Academy of Sciences, for the lending of this and other materials of *Coreocarpus* from the herbarium of that institution.

Coreocarpus sonoranus sp. nov.—Perennis, pallido-viridis, infra fruticosa divaricato-ramosaque supra herbacea adscendenti-ramosaque, sparsissime patenti-hispidula, ∓6 dm. alta, ramis tenuissimis elongatis superioribus subnudis. Folia bipinnatisecta petiolo tenuissimo 5–18 mm. longo, lamina 2–3.5 cm. longa, segmentis 1–2.5 (raro

–3) mm. latis saepius anguste oblongo-linearibus apicibus dentibusque subulatis. Capitula laxe disposita (vix subcorymbosa), tenuissime pedunculata (pedunculis 1–5 cm. longis, basi subulato-bracteatis), pansa ad anthesin 1.4–1.8 cm. lata et 5–6 mm. alta, cum fructibus circ. 7 vel 8 mm. crassa et circ. 4 mm. alta. Involucrum campanulatum vel late subcylindricum, bracteis oblongo-ovatis apice saepe subacuminatis demum 3–4 mm. longis. Flores ligulati plerumque 5, rosacei (praecipue secundum venas), tubo sparsim hispidulo, ligula oblonga vel lineari-oblanceolata apice denticulata 5–8 mm. longa. Paleae lineares vel oblongae, apice saepius acutae, disci floribus superatae sed achaenia superantes. Disci flores aurantiaci circ. 2.8 mm. longi. Achaenia exteriora corpore ipso (nigro, ventraliter et rarissime dorsaliter papillato-hispidulo) circ. 3 mm. longa et 1–1.2 mm. lata, alis plene in dentes nunc contiguos nunc separatos subbrunneos sub 1 mm. longos incisis, apice calvo; interiora angustiora.

Specimens examined: *Marcus E. Jones* 23365, Guaymas, State of Sonora, Mexico, Jan. 26, 1927 (type, Herb. Pomona College: cotypes, Herb. Gray; Herb. Univ. California).

I am indebted to Dr. Philip A. Munz, Professor of Botany, Pomona College, for the lending of this and other materials of *Coreopsis* from the herbarium of that institution.

Coreopsis stillmanii **jonesii** var. nov.—Herba ∓2 dm. alta, basi valde ramosa. Capitula pansa ad anthesin 3.5–4 cm. lata et circ. 1 cm. alta. Involucrum glabratum, bracteis exterioribus 8–16, tenuissime linearibus superne usque ad apicem obtuso-truncatum sensim angustatis, 7–12 mm. longis, demum saepe patentibus vel subreflexis, quam interioribus oblongo-ovatis paulo brevioribus. Achaenia corpore (ipso nigro et circ. 1 mm. lato) glaberrima vel secundum costam medianam sparsim papillato-hispidula alibi interdum paucissimis setulis (nunc acribus nunc terminaliter capitatis) munita, 4–4.5 mm. longa et (alis stramineis inclusis) circ. 2 mm. latis.

Specimens examined: *Marcus E. Jones* 3361 *pro parte*, Pasadena, California, May 2, 1882 (type, Herb. Pomona College).

With some, this would pass as a distinct species. In *C. douglasii* (a sister species), however, the latitude of variations in number, size, and shape of exterior involucral bracts is found to embrace the ex-

tremes represented (correspondingly) by *C. stillmanii* and var. *jonesii*. What is more, in *C. douglasii* these variations appear so often transitional that not even a varietal segregation seems justified.

COREOPSIS BORIANIANA Schz. Bip. *ex* Schweinf., Verhandl. Zool.-Bot. Ges. Wein **18**:684. 1868.—Recently, through the great kindness of DR. H. HUMBERT, Director of the Museum of Natural History of Paris, I have been lent the type of *Coreopsis chevalieri* O. Hoffm. & Muschl. (Bull. Soc. France 57, Mem. VIII, 118. 1910). This proves to be *C. borianiana* Schz. Bip. *ex* Schweinf.

Bidens brucei sp. nov.—Herba fruticosa, perennis, erecta, ∓ 1.8 m. alta, caulibus ramisque angulatis et non (nisi summam versus) pubescentibus. Folia opposita inferne in petiolos hispido-tomentulosos usque ad 1.5 cm. longos supra saepe marginatos abrupto-angustata, petiolo adjecto 6–11 cm. longa et 2–4.5 cm. lata, indivisa, lanceolato-oblonga vel saepius subrhomboideo-ovata, grosse serrata, apice subacuta, utrinque hispida. Capitula pauca, pedunculos subrobustos apicaliter folio-bracteatos terminantia, radiata, pansa ad anthesin circ. 7 cm. lata et circ. 1.5–1.8 cm. alta. Involucri hispidi bracteae 3–4-seriales, laxe adgregatae interdum patentes vel subreflexae subaequales, plus minusve oblongae, apice acutae, 1–2 cm. longae, intimae apicem versus angustatae. Flores ligulati 10–12, flavi, ligula oblongi vel lineares, apice parce denticulati, 3–4 cm. longi et 0.6–1.6 cm. lati. Paleae oblonge lineares, apice subacuto coloratae alibi stramineae 1.1–1.4 cm. longae. Achaenia late vel anguste oblongo-linearia, obcompressa, nigra, utraque facie circ. 8-sulculata, exalata, marginibus et facierum lineis perspicue erecteque papillato-setulosa, corpore 6–8 mm. longa et 1–2 mm. lata, apice erecte pauci-setosa et 2- (vel faciei ventralis costa mediana extensa saepe sub-3-)aristata aristis inferne robustis erecto-setosisque superne nudis tenuibusque ∓ 1 mm. longis.

Specimens examined: *Miss E. M. Bruce* 26, alt. 2200 ft., Ulugurue, Morogoro, Tanganyika Territory (German East Africa), Oct. 25, 1934 (type, Herb. Kew; *nom. vulg.*, m-luga); *B. D. Burtt* 4696, herb 3–4 ft. tall, on steep grass slopes, alt. 3000 ft., Uluguru Mts., above Morogoro, Tanganyika Territory, May 9, 1933 (Herb. Kew, 2 sheets).

BIDENS BRUCEI **pubescentior** var. nov.—A specie caulibus ramisque breviter patenti-hispidulis achaeniorum minorum (corpore 6–7 mm. longorum) apice numerose erecteque setoso aristis regulariter 2 tenuissimis ∓ 2 mm. longis non nisi base ipsa setosis differt.

Specimens examined: *G. B. Wallace* 294, herb. 6 ft. tall, alt. 3000 ft., Morogoro District, Tanganyika Territory, Feb. 16, 1932 (type, Herb. Kew; *nom. vulg.*, luzasu).

This species stands between *Bidens crataegifolia* (O. Hoffm.) Sherff and *B. coriacea* (O. Hoffm.) Sherff. I am indebted to SIR ARTHUR W. HILL, Director, and MISS E. A. BRUCE, Assistant Botanist, at the Royal Botanic Gardens of Kew, for the privilege of studying the original specimens. Related to these are now seen to be two hitherto enigmatic specimens collected some years ago by *Swynnerton* and named here:

BIDENS BRUCEI **swynnertonii** var. nov.—A var. *pubescentiore* achaeniis corpore 7–9 mm. longis et 2–2.2 mm. latis floribus ligulatis (tantum 8 pro singulo capitulo visis) forsan paucioribus differt.

Specimens examined: *C. F. M. Swynnerton* 859, Hiwaga, Tanganyika Territory, April–June, 1921 (type, Herb. Brit. Mus.); *idem* 860, Kigobcra, Tanganyika Territory, May–June, 1921 (Herb. Brit. Mus.).

BIDENS PILOSA var. RADIATA f. **dondiaefolia** (Less.) comb. nov.; *Bidens dondiaefolia* Less., Linnaea **5**:155. 1830.

BIDENS PILOSA var. RADIATA f. **decumbens** (Greenm.) comb. nov.; *Bidens decumbens* Greenm., Proc. Amer. Acad. **34**:576. 1899.

Bidens insolita sp. nov.—Verisimiliter perennis suberectaque; caulibus gracilibus, angulatis, glabratis, non nisi ad summam ramosis, ∓4 dm. altis Folia opposita, petiolata petiolis planis convexoconcavisve sparsim setoso-ciliatis sub 2 cm. longis, petiolo adjecto usque ad 1 dm. longa, bipinnatisecta, foliolis plerumque 5, saepe (segmentis linearibus apicaliter acerrimis membranaceis glaberrimis antrorsum spectantibus) subflabelliformibus. Capitula (4–8) subcorymbose disposita pedicellis tenuibus usque ad 8 cm. longis, radiata, pansa ad anthesin circ. 3–3.5 cm. lata et 8–10 mm. alta. Involucri bracteae exteriores plerumque 8, lineares, extus glabratae, marginibus albido-ciliatae, apice induratae et saepe acres, 4–5 mm.

longae, quam interiores ovato-oblongae apice pubescenti saepe abrupte angustatae breviores. Flores ligulati intense flavi, ∓1.5 cm. longi. Paleae lineares, superne angustatae, inferne interdum 1–3-dentatae dentibus erectis, 5–6 mm. longae. Flores tubulosi tenuissimi, corolla tubo brevi (circ. 1.5 mm.) adjecto demum circ. 5.5–6 mm. longa. Achaenia cuneato-linearia, subplana, brunneo-atra, utraque facie 4-sulcata, superne sparsim erecto-setulosa, corpore circ. 5–5.5 mm. longa et supra circ. 1 mm. lata, apice biaristata aristis flavidis retrorsum hamosis 2–3 mm. longis.

Specimens examined: *Howard Scott Gentry* 1971, meadow, Quicorichi, Rio Mayo, State of Chihuahua, Mexico, Oct. 7, 1935 (type, Herb. Field Mus.).

Bidens gentryi sp. nov.—Herba perennis, gracillima, circ. 1 m. alta; caulibus paucibus e unica radice, parce rectis sed non volubilibus, subteretibus, sulculatis, tantum circ. 1–1.3 mm. crassis. Folia opposita, petiolata petiolis tenuibus glabris plerumque 1–3 cm. longis, petiolo adjecto principalia 1–1.3 dm. longa, bipinnata vel tripinnatisecta; foliolis lateralibus primariis (imis tenuiter petiolulatis) 2 vel 3 jugis, membranaceissimis, glabratis vel margine sparsim setuloso-ciliatis, segmentis plus minusve lineari-oblongis vel ovato-lanceolatis terminali anguste attenuato; folioli terminalis segmento terminali angustissime lineari-acuminato usque ad 5.5 cm. longo. Capitula corymbose disposita (3 vel 4 ad caulis finem), pedunculata pedunculo tenui glabro ∓7 cm. longo, radiata, pansa ad anthesin circ. 3.8–4.3 cm. lata et ∓1.2 cm. alta. Involucri bracteae exteriores circ. 8, patentes vel subreflexae, lineares, apice acutae, glabratae, circ. 9–12 mm. longae; interiores oblongo-lanceolatae, non nisi apice pubescentes, breviores. Flores ligulati 7–9, flavi, ligula ellipticо-oblanceolati, apice saepe 2- vel 3-denticulati, ∓2 cm. longi. Paleae superne sensim angustatae, apice obtusae, sub 1 cm. longae. Achaenia submatura linearia, subtetragono-obcompressa, brunneo-atra; corpore glaberrimo vel apicem versus sparsissime erecto-setuloso, sub 1 cm. longo et sub 1 mm. crasso, omnibus (4) faciebus 2-sulculatis; aristis abortivis vel 2, tenuibus, sub 3 mm. longis, suberectis, retrorsum hamosis hamis paucis albidis acerrimis.

Specimens examined: *Howard Scott Gentry* 1700, growing a meter high, stalks several from the base, pine slope in Upper Sonoran re-

gion, Pinal, Sierra Charuco, State of Sonora, Mexico, Sept. 9, 1935 (type, Herb. Field Mus.).

Intermediate between *Bidens reptans* var. *urbanii* (Greenm.) O. E. Schulz of southern Mexico, Guatemala, and the West Indies and *B. urophylla* Sherff of Brazil. From the former it differs in its larger capitula, longer exterior involucral bracts, eciliate achenes, etc. From the latter it can be told at once by its more compound leaves, larger and more numerous ligulate florets, etc.

The last two species of *Bidens* and the following variety of *Cosmos* were among materials very kindly submitted to me by Dr. Paul C. Standley, Associate Curator of Botany at Field Museum of Natural History, Chicago.

Cosmos linearifolius **magnifolius** var. nov.—A specie foliis paucioribus majoribus principalibus 1–1.9 dm. longis et 6–10 mm. latis pedunculis usque ad 2.2 dm. longis differt.

Specimens examined: *Howard Scott Gentry* 1777, widely scattered, growing up along with grass, arid pine slopes, Arroyo Hondo, Sierra Charuco, State of Chihuahua, Mexico, Sept. 11, 1935 (type, Field Mus.).

Megalodonta beckii **oregonensis** var. nov.—A specie foliis submersis inferioribus (1–1.6 cm. longis) quam superioribus minoribus ac minus decompositis differt.

Specimens examined: *Frederick V. Coville* and *Elmer I. Applegate* 42, in the marsh at Buck Lake, yellow-pine woods, Klamath County, Oregon, Jul. 24, 1897 (2 type sheets in Herb. U.S. Nat.; cotype, Herb. Gray).

Not to be confused with *M. remota* Greene, from Green Lake, near Seattle, Washington, which latter species seems, from its two sheets of original material examined (*Piper* 1114, Herb. Gray), to represent *M. beckii* rather than my variety.

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TUSSOCK MEADOWS IN SOUTHEASTERN WISCONSIN

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 468

DAVID F. COSTELLO

(WITH NINE FIGURES)

Introduction

Throughout eastern Wisconsin, and extending eastward to the Atlantic seaboard, is a type of sedge meadow which seems to have been little studied and scarcely mentioned in the literature. It is characterized by numerous tussocks (figs. 1, 3), formed by sedges with the caespitose habit, which reach a height of 1–3 feet and which are separated by passages 1–4 feet wide. In bluegrass pastures the remnants of these tussocks are frequently represented by grass covered mounds a few inches in height. They almost invariably attract the attention of the traveler entering the region for the first time, but are taken for granted by the inhabitants, who usually explain their presence as being due to the trampling of livestock.

Although the floristic composition and the gross environment of these tussock meadows are fairly well known, ecological studies concerning the tussock habit, the floristic structure of the tussock association, and the factors which accompany and influence the initiation, development to maturity, and subsidence of these communities have been neglected. DRUDE (2) has briefly characterized similar associations in Hungary and has mentioned the gradual replacement of tussock meadows by grassland. WARMING and GRAEBNER (11) mention the formation of tussocks by *Carex paniculata* and *C. stricta* in Europe, and discuss at length the tussock vegetation of the subantarctic islands. They state that "tussock" signifies in general a vegetation of thick, high, bushlike tufts of grasses or grasslike plants. These tufts, composed principally of leaves, surmount compact pedestals which are formed from dead stems and leaf débris and which are ramified by numerous rhizomes and roots. The old pedestals frequently serve as substrata for other plants. NICHOLS

(6) states that the tussock sedge, *Carex stricta*, is "by far the commonest, and ecologically the most important, sedge of Connecticut swamps." He mentions its place in the usual swamp succession, lists a number of species characteristic of the sedge stage, calls attention to the composite habitat afforded by tussock meadows, and notes that the duration of the sedge stage may be long or short. In Wisconsin, STOUT (9) has studied the tussocks formed by *Carex stricta* in

FIG. 1.—*Carex stricta* tussock showing pedestal and tuft of leaves; *Ranunculus delphinifolius* on the ground.

connection with the other vegetation in a wild hay meadow. He gives statistical data concerning the frequence and abundance of the various species, but his work appears to deal with a mixed community rather than with a relatively pure stand of the tussock sedge. He describes the stooling habit of this sedge, discusses briefly its growth form and adaptations, and points out that the exclusiveness of *Carex stricta* is a problem that needs further study. In the introduction to his paper he includes a summary of the literature dealing with the geographic distribution of meadows and the classi-

fication of low moist formations. Although his paper does not deal primarily with the ecology of *Carex stricta*, it contains the best existing discussion of the species that has been written to date.

The present paper deals with the ecology of the tussock sedge, the structure of the tussock association, the place of the association in succession, and the environmental and biotic factors which accompany and influence the association throughout its life history.

The area included in this study comprises most of Ozaukee, Washington, Waukesha, Milwaukee, and Racine counties in Wisconsin. General observations were made of most of the meadow associations in this region within a radius of 35 miles from the city of Milwaukee. An area was selected for detailed study near the southern boundary of Ozaukee County, about 2 miles south of Cedarburg, on the west side of State Highway 57. The strip of meadow studied is somewhat over a quarter of a mile long and an eighth of a mile wide. The east side is low and moist, and consists principally of tussock meadow through which a small stream meanders. The west side rises gradually toward the top of a high ridge. Half way up this ridge, where the soil is kept moist by numerous springs, the tussock meadow again appears. Three-fourths of a mile northwest of this area an ephemeral pond with its surrounding tussock meadows was selected for further detailed study. Both of these areas are typical of scores of others that may be found within the same region.

Observations, beginning in 1927, were made over a period of six years. Detailed work was done in the field during the summers of 1929, 1932, and 1933. Studies of the growth habit of the tussock sedge were made during the winter as well as the summer.

Topographic and physiographic features

The areas considered in this paper all lie within the glaciated region of eastern Wisconsin. Consequently the topography is youthful, the relief is moderate, and the country is gently rolling. The topography is controlled by the Niagara cuesta (**5**), which is also spoken of as the eastern upland. The upland is underlain by Niagara limestone which dips eastward toward Lake Michigan. Covering the rock is a mantle of glacial drift of variable thickness, consisting principally of till, sand, gravel, and clay. In Waukesha and Wash-

ington counties the Kettle Moraine, an interlobate deposit, is characterized by greater irregularity and by pits or undrained depressions.

Temporary lakes and swampy tracts are common throughout the district. Many of these swampy areas are the result of filling of lakes; others are the result of seepage and poor drainage. According to WHITBECK (14), "Between twelve and fifteen per cent of this area is covered with lakes, swamps, or land that has been swampy." He also states that "not all of the present swamps were former lakes; probably the majority of them have been swamps from their beginnings." It is in these areas and in the drained depressions of the Kettle Moraine that tussock meadows have developed.

Methods

Floristic composition was studied by means of meter quadrats. Quadrats of this size were deemed necessary because of the uneven distribution of the vegetation. Transition areas and tension zones were studied by means of belt transects 0.5 and 1 m. wide. Immature plants were marked by stakes and later identified when flowers or fruit appeared. Doubtful plants were carefully pressed and later checked with authentic herbarium specimens. The presence and number of individuals and the coverage and height of each species within the quadrats were recorded. In order to facilitate counting, the plants were clipped from each quadrat. The culms of grasses and sedges were counted as individuals. Small plants such as *Eleocharis palustris* and *E. acicularis* were not counted but were rated for coverage and height.

In the study of the subterranean plant organs, trenches were dug to a depth greater than that of the deepest roots and the soil was then dissected away from the underground parts with an icepick or other sharp instrument. For more detailed studies of the roots in tussocks the whole pedestal was dug up and transported to the laboratory for dissection. Counts of the roots were made in winter when the frozen pedestal could easily be chopped off with an ax. For purposes of propagation, pieces of tussocks containing both roots and buds were brought into the greenhouse in winter and planted in soil or in water cultures.

Soil samples for moisture determination were taken weekly at depths of 6, 12, and 18 inches. The percentage of moisture was calculated on the basis of the dry weight of the soil.

Evaporation readings were taken from standardized porous porcelain atmometers. The usual precautions concerning rain absorption, the entrance of rain or dew into the bottles, and standardization of the bulbs were observed.

The level of the water table was determined throughout the growing season by weekly readings taken from numerous wells in typical parts of the area. These wells were plugged to reduce evaporation and to prevent seepage from rains or the accumulation of débris from the surface. At frequent intervals they were cleaned and then left undisturbed for at least 24 hours before a reading was taken.

The hydrogen ion concentration of the soil was determined in the field by means of the Morgan soil tester. These readings were checked in the laboratory on dried soil samples with the quinhydrone apparatus.

Vegetation of tussock meadow

Well developed tussock meadows are always dominated by a single species, *Carex stricta*. This plant frequently comprises more than 90 per cent of the vegetation and controls the physiognomy both in summer and in winter. In summer the deep green leaves obscure the pedestals beneath, but the presence of the tussocks is made apparent by the undulating nature of the sedge cover (fig. 2). In winter the hummocky nature of the area is most apparent (fig. 3). The dead leaves and culms, beaten down by wind and rain, form a close thatch about each pedestal.

It is only during the growing season that secondary species are at all conspicuous in the tussock association. In early spring these are apparent because of their showy flowers and the delayed development of the tussock sedge. The summer and autumn species are visible only because they surmount the general level of the meadow. Other species remain secluded beneath the sedge cover and are found only by pushing aside the tufts of leaves that grow from the tussocks.

Ecology of tussock sedge

Initiation and growth of tussocks.—New tussocks are initiated by rhizomes which grow down over the edge of the pedestal in the protection of the thatch of dead leaves, when the tussock is in the water, or which grow horizontally just beneath the soil when

Figs. 2, 3.—Fig. 2 (above), summer aspect of *Carex stricta* association; *Eupatorium* in foreground, *Typha* in background. Fig. 3 (below), well developed tussock association; water table about 4 inches below ground level.

the base of the tussock is a mere rounded hillock in drier situations. The pointed terminal buds of these rhizomes turn upward, producing a tuft of leaves, while roots develop from the base of the bud just beneath the soil. Under the most favorable conditions new tussocks are initiated in water that varies in depth from 6 to 12 inches. In a comparatively short time the leaves and culms appear above the

surface and act as a trap for floating débris, initiating the tussock almost as soon as the leaves appear.

New leaves appearing at the base of the young tuft throughout the growing season result in a well developed clump by the end of summer. Within a few years a pedestal consisting of dried culms, woody stems, old rhizomes, roots, soil, and vegetable matter is built up. Eventually the pedestals rise above the surface of the water (fig. 4), or during dry seasons they stand high and dry (fig. 1). In time

FIG. 4.—*Carex stricta* tussocks invading a pond

the mass of material which supports the clump of leaves becomes a network of fine fibrous roots which grow from the bases of the culms or develop as branches of larger wirelike roots that grow downward into the muck (fig. 9). From the crown of dead leaves above, long pointed buds grow out to produce new foliage each year.

If undisturbed, a tussock for several seasons may consist of a single plant. The culms and rootstocks remain so closely attached that they are dissected with difficulty. Eventually decay of the old woody parts below the surface may separate the stems, with the result that the tussock becomes a mass of interwoven but distinct individuals.

The age to which tussocks may attain is problematical. There is little doubt that under normal conditions they grow and develop for many years. Tussocks initiated in 1928 at the station south of

Cedarburg were 4 or 5 inches in diameter in 1934, but obviously these tussocks were then immature. Occasionally a vertical section through a tussock will show indications of layers of leaf bases and old stems that are deposited yearly. Calculations made on this basis would indicate an age of 40–60 years for some of the largest tussocks. Disregarding their destruction by violent means, by flooding, or by changes in the habitat, there is reason to believe that they may persist for half a century at least.

The rate of growth of larger tussocks is slow. Specimens marked for special study in permanent quadrats have shown almost no change in height or diameter over a period of 6 years. The maximum size to which they may ultimately attain depends upon the conditions under which the sedge grows. At the edges of ponds pedestals 2 feet in height with a diameter of 18 inches are not uncommon. From this size they grade down to small rounded mounds 2 inches in height and 6 inches in diameter.

The percentage of area covered by the bases of tussocks and the number of tussocks per unit area in different habitats are shown in table I. No correlation can be drawn between the percentage of area covered and the size of the tussock. The number of stools per unit area depends upon a great many factors, including the stage of development of the association, the degree of soil saturation, artificial drainage, annual fires, and grazing. In general, the larger the stools the smaller will be their number in a given area.

VEGETATIVE STRUCTURES OF TUSSOCK SEDGE.—The ability of *Carex stricta* to occupy certain areas in almost pure stands depends to a considerable extent upon the habits of the vegetative structures. Plants which at first appear to compete with the tussock sedge may be shown, by an investigation of root and rhizome habits, and of stratification above ground, to be adapted to different environments. The vegetative habits of *C. stricta* seem to offer at least a partial solution to this problem.

ROOT SYSTEM.—Two types of roots are present: long, cylindrical, wirelike soil roots which grow straight down into the substratum and slender, tortuous, much branched, fibrous roots which permeate the earthy pedestal of the tussock. The soil roots arise from the sides of the culms or from the short compressed internodal regions

of the woody stem which lie just beneath the surface of the tussock (fig. 9). They are of rather uniform diameter, varying from 2 mm. in thickness at the point of origin to 0.5 mm. a short distance back from the tip. They range in length from 1.5 to 7 dm., depending upon the nature of the substratum, compactness of the soil, water table, and moisture content of the soil. Within the pedestal, especially when aeration is good, they may branch freely, with the result that a matted spongy mass is formed. Below the general soil level branching is less pronounced. This is especially apparent in winter when

TABLE I

COMPARISON OF SIZE AND NUMBER OF TUSSOCKS, AND PERCENTAGE OF AREA COVERED BY THEIR BASES

HABITAT	AV. DIAMETER OF TUSSOCK (DM.)	NO. OF DIAMETER QUADRATS	AV. NO. OF TUSSOCKS PER SQ. METER	PERCENTAGE AREA COVERED BY TUSSOCK BASES
Permanent pond	3.0	64	1.5	10.6
Intermittent pond	3.2	43	2.3	18.5
Morainic depression	3.5	55	1.9	18.2
Floodplain	3.0	80	3.6	25.4
Springy hillside	3.1	35	4.1	10.9
Margin of stream	4.5	32	1.6	15.9
Pond margin	3.6	28	4.2	42.7
Floodplain (burned)	1.5	40	5.0	8.8
Swale	5.0	100	0.9	17.6

the frozen hummock is chopped or broken off; the soil roots then project from the exposed surface like so many stiff wires.

The number of vertical soil roots within the pedestal is considerable. A cross section of a pedestal 1.5 dm. in diameter will show from 250 to 400 roots; larger tussocks will show a correspondingly greater number. The largest tussock examined was slightly over 8 dm. in diameter and showed approximately 3200 roots.

The depth to which the soil roots will penetrate is determined to a considerable extent by the ground-water level. In areas where the water stands continuously above the soil the root system is shallow. If the water table is fluctuating the roots may penetrate to the lowest level of the water table, a distance seldom more than 24 inches below the soil surface.

Root hairs are produced to a limited extent by the periodically submerged soil roots. If the soil is compact and clayey they may be entirely absent. Root hairs are produced in great abundance by soil roots, however, which frequently grow down the sides of the pedestal or which become exposed to the air by the lowering of the water in ponds. A thick feltlike covering is formed by the numerous hairs, which gradually lignify and turn brown. In the greenhouse they may be produced on plants grown in water culture by gradually lowering the water level, or by growing the plants in well aerated soil which is watered sparingly. Whether or not these root hairs possess any power of absorbing moisture from the air has not been determined.

RHIZOME SYSTEM.—Vegetative propagation of *Carex stricta* is accomplished by means of rhizomes. The rootstocks arise from the bases of the culms, and as STOUT (9) has pointed out, are of two types: the ascending and the creeping.

The terminal buds of the ascending rhizomes immediately give rise to new leafy shoots and thus increase the height and lateral spread of the tussock. The creeping rhizomes extend a few inches beneath the soil until they reach a length of a foot or more. The terminal bud may then turn upward to produce a shoot and initiate a tussock. Frequently the rhizomes of tall tussocks are bent over the edge of the pedestal, half concealed in the thatch of dead leaves, their long pointed buds directed toward the ground (fig. 9). Occasionally they grow downward through the mass of roots and then horizontally a short distance below the surface of the soil.

Carex stricta is not so strongly stoloniferous as *C. strictior*, which grows in somewhat similar situations and occasionally with the tussock sedge. *C. strictior*, however, does not produce distinctive tussocks but forms large flat clumps. Although the two species are sometimes distinguished with difficulty in herbaria, there can be no doubt of their identity in the field. MACKENZIE (4) has pointed out the taxonomic features of these two sedges, including the nature of the stolons.

While the ascending stolons provide both for leaf and flower production, it is apparent that the tussock sedge depends almost exclusively upon vegetative growth for its maintenance. Examination

of thousands of tussocks over a period of more than six years in the Milwaukee region has yielded comparatively few fruiting specimens. Seedlings of *Carex stricta* were never found during this period.

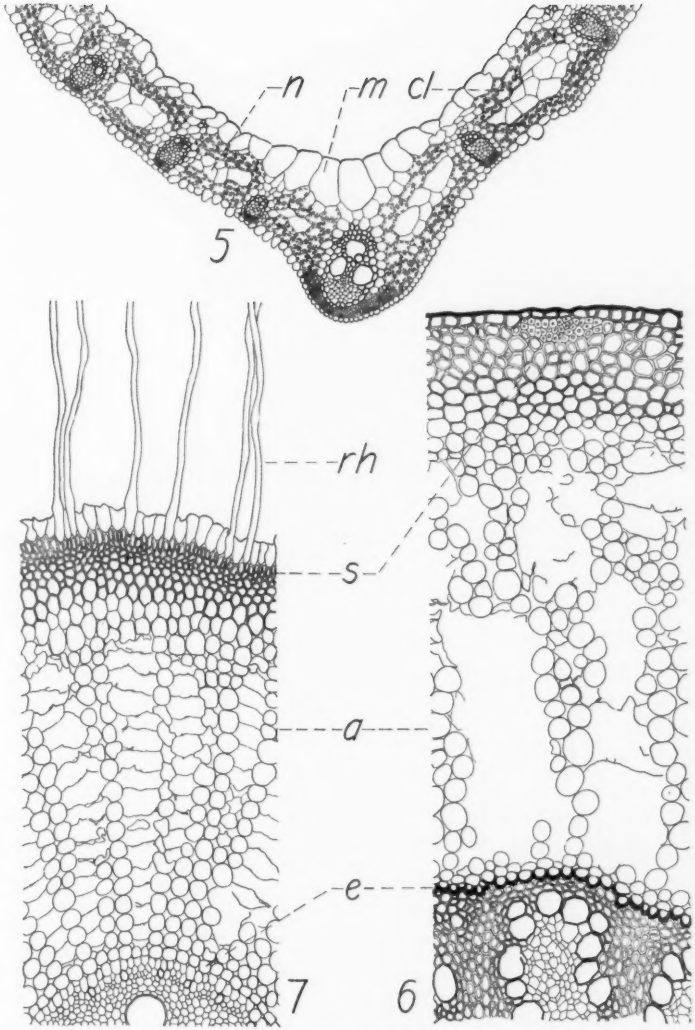
The leaves form a close fascicle as they arise from the ascending stolon; hence in the quadrat studies leaf clusters or culms were counted rather than individual leaves. A tussock 10 inches in diameter will ordinarily show from 100 to 150 clusters. When fully mature the leaves form a dense cover, shading even the open spaces between the tussocks. In early summer the aspect is that of a luxuriant meadow, all evidence of the uneven nature of the ground frequently being obscured.

ECOLOGICAL ANATOMY.—The leaves of *Carex stricta* present a comparatively small area of exposed surface. Although they vary in length from 4 dm. to more than 1 m., they are seldom more than 7 or 8 mm. wide and are usually dried at the tips. When subjected to drought they fold upward on the midrib, but the blades are stiff enough to resist twisting by the wind.

In cross section the leaf is seen to consist of a series of parallel bundles separated by compartments of chlorenchyma (fig. 5). The bundles are surrounded by sclerenchymatous sheaths which are in turn surrounded by non-chlorophyll bearing parenchymatous cells. The chlorenchyma usually surrounds a group of non-chlorophyll bearing cells. In older leaves the latter may break down to form lacunae. The upper and lower epidermis possesses a cuticle of medium thickness. Bulliform or motor cells occur in the groove of the ventral (adaxial) side of the leaf. They provide for the folding of the leaf by their collapse during periods of desiccation.

The leaves of *Carex stricta* are well adapted to withstand high evaporation and the periods of summer drought to which they are sometimes subjected, although they do not show the pronounced xeromorphic structure which is characteristic of some of the upland species of grasses (13). The persistence of the tussock sedge in dry, well grazed pastures may be explained in part by its semi-xeromorphic leaf structure.

The roots and rhizomes show both hydromorphic and xeromorphic structure. HAYDEN (3) has pointed out that the primary indicators of water relations in underground organs are the relative proportions



FIGS. 5–7.—Transverse sections of leaf, rhizome, and root of *Carex stricta* showing ecological anatomy: fig. 5, transection of leaf (*cl*, chlorenchyma; *m*, motor cells; *n*, non-chlorophyll bearing cells); fig. 6, transection of mature rhizome; fig. 7, transection of young root (*e*, endodermis; *a*, aerenchyma; *s*, sclerenchyma; *rh*, root hairs).

of parenchyma, mechanical tissue, and conductive tissue. Xeric structure is indicated by the prominence of the latter two, and the reverse condition is indicated by the first.

In the rootstock (fig. 6) the cortical tissue occupies two-thirds of the radius of the rhizome. The thick walled epidermis surrounds a sclerenchyma layer which is six to eight cells in thickness. The zone of aerenchyma is pronounced and is characterized by large lacunae. A prominent endodermis with thickened radial and inner tangential walls is present. The stele is a close fascicle of numerous bundles, each surrounded by a compact sheath, inclosing a small pith cylinder of thin walled aerenchyma.

In the root (fig. 7) the cortical area represents from two-thirds to three-fourths the diameter of the entire structure. Numerous lignified root hairs may or may not be present. The sclerenchyma layer beneath varies from five to eight cells in thickness. Aerenchyma is even more pronounced here than in the rhizome. The radial and inner tangential walls of the endodermis are strongly thickened. The stele is composed of a single row of bundles surrounding a pith cylinder of lignified cells.

The prominence of mechanical tissue in both the root and the rhizome would seem to indicate xerophytism, while the abundance of aerenchyma indicates hydric tendencies. This condition, however, does not appear to be anomalous when the water relations of *Carex stricta* are considered. As a rule both roots and rhizomes are exposed to the atmosphere for a portion of each growing season. Adequate protection from desiccation is afforded by a sclerenchyma layer in the outer part of the cortex. At other times the root and rhizome systems are submerged. The utility of an aerating system then becomes apparent. The presence of additional mechanical tissue in the stele explains to some extent the difficulty with which tussocks are uprooted or toppled over, either by artificial means or as the result of natural forces.

TUSSOCK ASSOCIATION

The vegetation of the tussock meadow is almost exclusively herbaceous. *Carex stricta* is the dominant of the association. Other sedges occur, particularly *C. bebbii*, *C. hystericina*, *C. vulpinoidea*,

and *C. lanuginosa*, but never in sufficient abundance to change markedly the floristic composition of the area. When present they are generally limited to the mesic side of the association. The more mesophytic grasses, *Calamagrostis canadensis*, *Glyceria nervata*, *Bromus ciliatus*, *Agrostis alba*, and *Phragmites communis*, occasionally change the appearance of the association late in the season owing to the height of their culms, which overtop the other species, or owing to contrast in the color of their foliage. Few species deserve to be designated as constants. *Asclepias incarnata*, *Lycopus americanus*, and *Equisetum arvense* appear in a high percentage of the stands but they may be totally absent. In late summer and early autumn *Eupatorium maculatum*, *E. perfoliatum*, *Solidago* spp., *Aster* spp., *Stachys tenuifolia*, and *Bidens frondosa* may be found in stands of considerable extent. Some of the conspicuous but ecologically unimportant species which occasionally appear are *Iris virginica*, *Symplocarpus foetidus*, *Caltha palustris*, *Lilium michiganense*, *Hemerocallis fulva*, *Spiraea alba*, and *Lobelia siphilitica*.

The composite nature of the habitat has been pointed out by Nichols (**6**). He mentions a few of the hydrophytic species which are able to grow in the moist substratum between the tussocks and a number of plants which are able to thrive in the drier substratum of the tussock itself. Shade tolerant species are especially characteristic of the inter-tussock vegetation. In general, species which propagate themselves mainly by vegetative means are found in the spaces between the tussocks. Typical species are *Equisetum arvense*, *Impatiens biflora*, *Marchantia polymorpha*, *Conocephalum conicum*, and *Typha latifolia*. Species which spread readily by seed commonly grow from the tops of the tussocks. *Bidens frondosa* is probably the best example, especially on tussocks growing in ponds. In early summer its seedlings may be found in the crowns of the tussocks. As the season progresses the tap roots grow down the sides of the pedestals, apparently keeping pace with the lowering water table. In well grazed pastures where *Carex stricta* persists only as a relict, *Verbena hastata* is almost invariably present in the crowns of the tussocks.

Woody forms are represented by various willows, especially *Salix discolor*, *S. petiolaris*, and *S. interior*. Occasionally *Spiraea alba*,

Cornus stolonifera, and *Rhamnus alnifolia* appear as isolated individuals in the meadow. More commonly the willows appear *en masse* at the margin of the association, where they represent the stage in succession that is to follow.

A statistical analysis of four stands is shown in table II. Stand no. 1 borders a small pond; it is about 3 acres in extent. Stand no. 2 covers the floodplain of a small stream, the ground sloping gradually upward on either side of the stream; it covers an area of approximately 15 acres. Stand no. 3 lies in a drained depression of the Kettle Moraine; it has an area of 4 acres. Stand no. 4 lies on an east facing slope characterized by seepage and underground springs; it occupies about three-fourths of an acre.

In table II the numbers or symbols in the columns have the following significance: constancy is indicated by the number preceding each period; vegetational cover is indicated by the number following. Species of infrequent occurrence are represented by a plus sign. When the species is absent from the stand this is indicated by a minus sign. Presence and cover are expressed in five degrees as follows (1):

Presence:

1, present in less than $\frac{1}{5}$ of quadrats studied
2, present in $\frac{1}{5}$ to $\frac{2}{5}$ of quadrats studied
3, present in $\frac{2}{5}$ to $\frac{3}{5}$ of quadrats studied
4, present in $\frac{3}{5}$ to $\frac{4}{5}$ of quadrats studied
5, present in $\frac{4}{5}$ to $\frac{5}{5}$ of quadrats studied

Cover:

1, covering feeble, less than $\frac{1}{20}$ of area
2, covering $\frac{1}{20}$ to $\frac{1}{4}$ of area
3, covering $\frac{1}{4}$ to $\frac{1}{2}$ of area
4, covering $\frac{1}{2}$ to $\frac{3}{4}$ of area
5, covering more than $\frac{3}{4}$ of area

COMPETITION AND ADAPTATION WITHIN THE ASSOCIATION.—A plant association may be more than a collection of species adapted in the same or in different fashion to similar conditions of habitat (9). It may include species which are not adapted and species which are so aggressive or capable of occupying the area that their presence

TABLE II

TUSSOCK ASSOCIATIONS

NUMBER OF STAND	1	2	3	4
SIZE OF QUADRAT	1 SQ. M.	1 SQ. M.	1 SQ. M.	1 SQ. M.
QUADRATS SCORED	12	20	18	24
VEGETATION COVER %	70	100	100	100
TYPE OF HABITAT	BORDER OF POND	FLOOD-PLAIN	DEPRES-SION	HILLSIDE (SPRINGS)
Carex stricta	5 . 3	5 . 5	5 . 5	5 . 5
Solidago spp.	1 . 1	3 . 2	3 . 1	1 . 1
Equisetum arvense	+	2 . 1	3 . 1	−
Impatiens biflora	2 . 1	1 . 1	1 . 1	1 . 1
Eupatorium maculatum	+	2 . 2	1 . 1	+
E. perfoliatum	+	2 . 2	+	+
Asclepias incarnata	1 . 1	2 . 1	+	−
Bidens frondosa	3 . 2	−	−	−
Galium asprellum	−	3 . 2	−	−
Typha latifolia	−	1 . 1	−	−
Salix petiolaris	1 . 1	1 . 1	−	−
S. interior	1 . 1	−	−	−
S. bebbiana	1 . 1	−	−	−
Caltha palustris	1 . 1	+	+	−
Iris virginica	1 . 1	−	−	−
Scirpus palustris	1 . 1	−	−	−
Rumex verticillatus	+	+	1 . 1	−
Stachys tenuifolia	−	+	2 . 2	−
Ambrosia artaemisiifolia	−	−	1 . 1	−
Viola sp.	−	1 . 1	−	−
Calamagrostis canadensis	−	−	1 . 1	−
Marchantia polymorpha	−	1 . 2	−	−
Conocephalum conicum	−	−	−	1 . 1
Barbula unguiculata	−	1 . 1	−	−
Symplocarpus foetidus	−	−	−	1 . 3
Hemerocallis fulva	−	+	−	−
Lilium michiganense	−	+	−	−
Smilacina stellata	−	−	−	1 . 1
Carex lanuginosa	−	1 . 1	−	−
Thalictrum dasycarpum	−	1 . 1	−	−
Spiraea alba	−	+	−	−
Verbena hastata	−	−	−	1 . 1
Campanula aparinoides	−	1 . 1	−	−
Aster novae-angliae	−	1 . 1	−	−
Lycopus americanus	−	2 . 1	1 . 1	−
Carex retrorsa	+	−	−	−
Carex spp.	+	+	+	+
Lysimachia thyrsiflora	+	−	−	−
Carex riparia	1 . 1	−	−	−
Trifolium procumbens	−	−	+	−
Solanum dulcamara	−	−	−	+
Chelone glabra	−	+	−	−
Lobelia siphilitica	−	−	−	+
Helianthus grosseserratus	−	+	−	−

is detrimental to the characteristic species. SHERFF (8) has emphasized two types of relationships which may exist within a community: the complementary, in which the plant organs grow and vegetate at different levels; and the competitive, in which the plant members occupy the same stratum. These relationships will be considered now from the standpoint of the aerial and of the subterranean plant organs.

AERIAL RELATIONSHIPS.—Among the complementary species of the association are those plants which show a marked seasonal adaptation. An examination of the tussock meadow in early spring shows a number of species which flower and fruit long before the dense growth of sedges has formed a closed cover. There are many shallow-rooting species present which become inconspicuous or disappear entirely with the advent of summer. All of these grow in the spaces between the tussocks where there is an abundance of moisture and light during the early growing season. By June 15 they are usually overtopped by the sedges.

In late summer and early autumn a considerable number of species appear which exceed the general meadow cover in height but which may be still considered mostly complementary in their aerial relationships. The period of flowering and fruiting comes after the typical meadow vegetation has ceased to grow vigorously or has even become dry. *Eupatorium purpureum*, *Solidago serotina*, *Helenium autumnale*, and *Aster novae-angliae* may be placed in this group.

Stratification is not especially marked in the tussock meadow, although a number of species present show a definite light or moisture requirement. Data obtained from atmometer studies (see later paragraphs) in these meadows conform to the findings of YAPP (15) that the evaporation rate rapidly increases from the ground upward. There is little doubt that the presence of mosses and liverworts at the lowest level may be correlated with the abundance of moisture, the higher humidity, and the dense shade. Among the species which grow in the shade of the tussock sedge are *Impatiens biflora*, *Ambrosia artemisiifolia*, *Equisetum arvense*, *Dryopteris thelypteris*, *Trifolium procumbens*, *Pilea pumila*, and seedlings of

Xanthium sp. Owing to their relatively weak growth, none of these may be considered as aerial competitors of the sedge.

Among the competitive species of greatest importance are those which grow concurrently with the dominant vegetation. Most of the sedges and grasses of the association belong to this group. Of these, *Calamagrostis canadensis* is probably the most important. In the drier portions of a tussock meadow the culms of this grass may occasionally equal or exceed in number those of *Carex stricta*. In late summer the grass gains the ascendancy over the sedge and may even obscure the latter in dense stands. In the moister areas, however, *Calamagrostis* loses its aggressiveness and appears only in scattered bunches, if it is present at all. Of lesser importance are the sedges, *Carex vulpinoidea*, *C. hystericina*, *C. lanuginosa*, and *C. stipata*, and the grasses *Phragmites communis*, *Bromus ciliatus*, *Spartina pectinata*, *Leersia oryzoides*, and *Glyceria nervata*. Other species such as *Scirpus atrovirens* and *S. lineatus* are of such infrequent occurrence that they are of little importance as competitors.

A number of species which may be considered as strongly competitive are those which commonly grow on the tops of the tussocks. DRUDE (2) states in his description of *Carex stricta* meadows in Hungary that a motley array of plants establish themselves on the tops of the tussocks even when they are still in ponds. However, he mentions specifically only the thistles and orchids. NICHOLS (6) mentions a number of plants that grow on the tussocks in Connecticut swamps. In general, plants which reproduce by seeds appear to be the most frequent invaders of the tussocks themselves. At best these intruders find themselves in uncongenial surroundings. They have to contend with the overwhelming numbers of sedge leaves; their roots must penetrate a tangled substratum of dead culms, rhizomes, and matted rootlets; and not infrequently the top of the tussock dries out before the young seedlings have established themselves.

One of the most successful of these invaders is *Bidens frondosa*. It begins its growth in the middle of summer and attains its greatest luxuriance long after the sedges have reached their maturity. It reaches its greatest abundance in areas which are shaded by taller

species, particularly the willows. Its presence in such situations is a good indication that the tussock association is disappearing.

SUBTERRANEAN RELATIONSHIPS.—An examination of the root system of *Carex stricta* reveals the fact that the greatest competition in the tussock meadow is not above but below the surface of the soil. It explains to a certain extent the ability of the tussock sedge to occupy the habitat almost to the exclusion of everything else. Aided by its aerating system, the sedge permeates the saturated soil with roots and rhizomes. The interrelationships of the subterranean organs of the tussock sedge with other species and their possible significance will be considered now.

SAGITTARIA.—This plant, particularly in ponds being invaded by *Carex stricta*, frequently forms a border on the hydric side of the tussock association. It almost never mingles with the tussocks. The transition from the *Sagittaria* zone to tussock meadow is abrupt. SHERFF (**8**) has pointed out that the habit of growth of *Sagittaria* does not favor a compact grouping of individuals, since the rhizomes are not of the mat producing type. It can be invaded easily by *C. stricta*. On the other hand, although rhizomes of *Sagittaria* grow to a depth of 15 cm., they cannot grow beneath the deeper root system of the sedge. Thus it appears that the sedge is limited in its advance on the pond not by the zone of *Sagittaria* but by its own ability to grow in deep water.

SPARGANIUM AND TYPHA.—These frequently border the tussock association on the hydric side, especially along streams and occasionally in ponds. Relict stands of *Typha* are sometimes found in the midst of an otherwise well developed tussock meadow. Root and rhizome competition is always marked between these species. The densely matting rhizomes of both genera are instrumental in maintaining almost pure stands, once they have become established. In addition, their compact habit of growth excludes *Carex stricta*, until the building up of soil and the consequent lowering of the water table so modify habitat conditions that the aggressiveness of *Sparganium* and *Typha* disappears.

CAREX RIPARIA.—Dense stands of this rank growing sedge are sometimes found in the tussock association, although they are not a part of it. These stands may occur on the hydric side, the mesic

side (as islands within the association), or as broad bands running completely through the association. Superficial examination discloses no very great variation in the habitat. The area occupied by *Carex riparia* apparently should support a luxuriant tussock meadow. The explanation rests upon a number of factors.

The rootstocks of *C. riparia* are very coarse and are capable of rapid growth. From the base of the culm they grow downward, and at a depth varying from 5 to 20 cm. they assume a horizontal direction for a distance of 10 to 50 cm.; the tips then grow vertically to give rise to a new plant at the surface of the ground. Roots are produced both from the base of the culm and from the nodes of the rootstock. Thus the entire soil layer to a depth of 15–20 cm. is permeated with a coarse mat of subterranean organs. Growth of the rhizomes is very rapid in the loose spongy soil.

The areas occupied by *C. riparia* are almost invariably flooded by débris and soil washed from surrounding uplands. The accumulated soil may amount to 6 inches or more within a single season. Owing to the rapid growth of rhizomes, *C. riparia* keeps pace with the rising soil level with ease. The culms are numerous, 150 or more per square meter, and because of their rank growth tend to overshadow all other plants in the same area. Hence this species occupies space to the exclusion of everything else. Under these conditions the slower growing *C. stricta* is soon covered with soil and the invasion of *C. riparia* is quickly accomplished.

SPECIES WITH SHALLOW ROOT SYSTEMS.—Throughout the association are numerous species which produce roots at or near the surface of the soil. In relation to the tussock sedge they are entirely complementary. In ponds the runners of *Ranunculus delphinifolius* frequently cover the soil surface following recession of the water (fig. 1). In the more elevated portions of the association *Asclepias incarnata*, *Penthorum sedoides*, *Echinochloa crusgalli*, *Lycopus americanus*, and *Galium* sp. may occur. They seem to offer little competition to the tussock sedge.

LIST OF SPECIES.—The species found to occur in quadrats studied in the tussock association of the five counties previously mentioned are enumerated in the following list. The numbers preceding the species indicate their usual position in the association as follows:

(1) on the hydric side, (2) in the region of maximum development, and (3) on the mesic side or in transition areas. In general the nomenclature follows that of GRAY'S manual (7); recent changes and segregations in certain genera, however, have been observed.

Species occurring in tussock association

Musci

(2) Barbula unguiculata (Huds.) Hedw.
(2) Brachythecium rivulare B. & S.
(1) Drepanocladus aduncus pseudofluitans Sanio
(3) Bryum sp.

Hepaticae

(1, 2) Marchantia polymorpha L.
(1, 2) Conocephalum conicum (L.) Dum.

Polypodiaceae

(3) Dryopteris thelypteris (L.) Gray

Equisetaceae

(2, 3) Equisetum arvense L.

Typhaceae

(1, 2) Typha latifolia L.

Sparganiaceae

(1) Sparganium eurycarpum Engelm.

Alismaceae

(1) Sagittaria latifolia Willd.
(1) Alisma plantago-aquatica L.

Gramineae

(3) Echinochloa crusgalli (L.) Beauv.
(3) Zizania aquatica L.
(3) Leersia oryzoides (L.) Sw.
(3) Phalaris arundinacea L.
(3) Muhlenbergia racemosa (Michx.) B.S.P.
(3) Phleum pratense L.
(3) Agrostis alba L.
(2, 3) Calamagrostis canadensis (Michx.) Beauv.
(3) C. neglecta (Ehrh.) Gaertn.
(3) Koeleria cristata Pers.

(3) Spartina pectinata Link (S. michauxiana Hitchc.)
(1) Phragmites communis Trin.
(3) Poa pratensis L.
(1) Glyceria nervata (Willd.) Trin.
(2, 3) Bromus ciliatus L.
(3) Hordeum jubatum L.
(3) Elymus canadensis L.

Cyperaceae

(3) Eleocharis obtusa (Willd.) Schultes
(1, 2, 3) E. palustris (L.) R. & S.
(3) E. acicularis (L.) R. & S.
(1) Scirpus fluviatilis Gray
(3) S. atrovirens Muhl.
(3) S. lineatus Michx.
(3) Carex bebbii Olney
(3) C. sterilis Willd.
(3) C. bromoides Schkuhr.
(3) C. vulpinoidea Michx.
(3) C. stipata Muhl.
(3) C. sartwellii Dewey
C. stricta Lam.
(3) C. strictior Dewey
(3) C. pennsylvanica Lam.
(2, 3) C. lanuginosa Michx.
(3) C. trichocarpa Muhl.
(1, 2, 3) C. riparia W. Curtis
(3) C. hystericina Muhl.
(1) C. retrorsa Schw.
(2, 3) C. rostrata Stokes

Araceae

(2) Symplocarpus foetidus (L.) Nutt.

Lemnaceae

(1) Lemna trisculca L.

Juncaceae

(3) Juncus tenuis Willd.
(3) J. dudleyi Wieg.

Liliaceae

(2) Hemerocallis fulva L.
(3) Lilium michiganense Farwell
(2) Smilacina stellata (L.) Desf.

Iridaceae

(1) Iris virginica L.
(3) Sisyrinchium gramineum Curtis

Salicaceae

(3) Salix nigra Marsh.
(1, 2, 3) S. amygdaloides Anders.
(1) S. lucida Muhl.
(1, 2, 3) S. interior Rowlee (S. longifolia Muhl.)
(1, 2, 3) S. cordata Muhl.
(1, 2, 3) S. discolor Muhl.
(1, 2, 3) S. petiolaris Smith
(1, 2, 3) S. bebbiana Sarg. (S. rostrata Richards)
(2) S. candida Flügge

Urticaceae

(3) Urtica procera Muhl. (U. gracilis American authors)
(3) Laportea canadensis (L.) Gaud.
(2) Pilea pumila (L.) Gray
(3) Boehmeria cylindrica (L.) Sw.

Polygonaceae

(3) Rumex crispus L.
(2, 3) R. verticillatus L.
(1) Polygonum sp.

Ranunculaceae

(1) Ranunculus delphinifolius Torr.
(3) R. abortivus L.
(2) R. pennsylvanicus L.f.
(2, 3) Thalictrum dasycarpum Fich. & Lall.
(3) Anemone virginiana L.
(3) A. canadensis L.
(1, 2) Caltha palustris L.

Cruciferae

(3) Cardamine bulbosa (Schreb.) B.S.P.
(3) C. douglassii (Torr.) Britton

Crassulaceae
(3) Penthorum sedoides L.
Saxifragaceae
(3) Saxifraga pennsylvanica L.
(3) Parnassia caroliniana Michx.
Rosaceae
(2) Spiraea alba DuRoi
(3) Potentilla monspeliensis L.
(2, 3) Rosa carolina L.
Leguminosae
(2) Trifolium procumbens L.
(3) Lathyrus palustris L.
(3) Apios tuberosa Moench.
Callitrichaceae
(1) Callitriche palustris L.
Balsaminaceae
(1, 2, 3) Impatiens biflora Walt.
Rhamnaceae
(2) Rhamnus alnifolia L'Her.
Violaceae
(2, 3) Viola cucullata Ait.
(2) V. blanda Willd.
Onagraceae
(2) Epilobium densum Raf.
Umbelliferae
(1) Cicuta maculata L.
(1) Sium suave Walt. (S. cicutaefolium Schrank.)
(3) Zizia aurea (L.) Koch
(2) Pastinaca sativa L.
(3) Oxypolis rigidior (L.) Coult. & Rose
(3) Angelica atropurpurea L.
Primulaceae
(1) Lysimachia thyrsiflora L.
Gentianaceae
(3) Gentiana andrewsii Griseb.
Asclepiadaceae
(1, 2) Asclepias incarnata L.
(3) A. syriaca L.

Verbenaceae
(3) Verbena hastata L.
Labiatae
(1) Scutellaria lateriflora L.
(1) S. galericulata L.
(3) Prunella vulgaris L.
(2) Physostegia virginiana (L.) Benth.
(3) Stachys tenuifolia var. aspera (Michx.) Fernald
(2, 3) Lycopus virginicus L.
(2, 3) L. americanus Muhl.
(3) Mentha arvensis var. canadensis (L.) Briquet
Solanaceae
(3) Solanum dulcamara L.
Scrophulariaceae
(3) Linaria vulgaris Hill.
(3) Scrophularia marilandica L.
(2) Chelone glabra var. linifolia Coleman
(3) Mimulus ringens L.
Plantaginaceae
(3) Plantago major L.
Rubiaceae
(2) Galium asprellum Michx.
Campanulaceae
(2, 3) Campanula aparinoides Pursh
Lobeliaceae
(2, 3) Lobelia syphilitica L.
Compositae
(2, 3) Eupatorium purpureum L.
(2, 3) E. perfoliatum L.
(3) Solidago canadensis L.
(3) S. serotina Ait.
(2, 3) Aster novae-angliae L.
(2, 3) A. puniceus L.
(3) Ambrosia artemisiifolia L.
(3) Xanthium sp.
(3) Rudbeckia hirta L.
(3) Helianthus grosseserratus Martens

(1) Bidens frondosa L.
(3) B. connata Muhl.
(3) Helenium autumnale L.
(3) Cirsium lanceolatum (L.) Hill

Succession

The tussock meadows in these areas constitute a stage in succession which is usually preceded by a reed-swamp stage and followed by the *Eleocharis-Juncus-Carex* complex of the typical hydrosere. The limits of the sedge meadow are usually well marked, owing to the growth form of its principal species, *Carex stricta*. The transition from open water (fig. 4) or from the reed-swamp stage to the tussock meadow is usually abrupt. Telescoping is occasionally apparent if the habitat conditions are changing rapidly or the topography is variable within the area. The tension zone along streams may be even more marked if the land rises gradually on both sides of the stream. In flat areas the association may be uniform throughout, although islands of relicts from preceding stages or of invaders indicating the stages to follow are occasionally present.

The transition to the following stage is usually a gradual one and is indicated more by the decreasing numbers of the tussocks than by the appearance of new or otherwise conspicuous species. The outer limits of the association are especially well marked in areas where rising ground is accompanied by a rapid increase in depth of the water table, in situations marked by abrupt changes in the soil type, and in grazed areas. Heavy pasturage may result in grassland directly bordering the tussock meadow. In all cases the boundary of the association may be determined by the presence or absence of the tussock sedge.

The *Eleocharis-Juncus-Carex* complex which usually succeeds the tussock association is dominantly herbaceous. The most characteristic species are *Eleocharis palustris*, *Juncus dudleyi*, and *Carex bebbii*. The less frequent *Scirpus atrovirens* is usually present and is rendered conspicuous by its coarser growth and greater height. Intermingled with these species are various sedges and grasses: *Carex vulpinoidea*, *C. sartwellii*, *C. hystericina*, *Bromus ciliatus*, *Leersia*

oryzoides, *Glyceria nervata*, and *Calamagrostis canadensis*. A considerable array of dicotyledonous forms appears throughout the season. If the area is not subjected to heavy grazing, *Rumex crispus*, *Ranunculus abortivus*, *Penthorum sedoides*, *Saxifaga pennsylvanica*, *Lathyrus palustris*, *Mentha arvensis*, *Lycopus americanus*, *Mimulus ringens*, and *Lobelia syphilitica* are usually present. The autumnal aspect is usually characterized by a display of *Aster* and *Solidago* spp. which may completely obscure the meadow-like appearance of the complex.

In the tension zone between the tussock association and the *Eleocharis-Juncus-Carex* complex, *Carex stricta* usually shows a definite change in growth form. The pedestals become low mounds or merely tufts of culms growing at the soil level. Occasionally the spaces between the tufts become occupied by culms that have grown from rhizomes and a uniform distribution results. At this stage the tussock sedge is sometimes difficult to distinguish from the closely related *C. strictior*, which grows in mats rather than in tussocks. The deep green leaves of the former, however, serve to distinguish it from the paler glaucous foliage of the latter. By digging up the root system of *Carex stricta* in such places the remains of buried tussocks can sometimes be found.

In areas not subjected to grazing, succession may proceed through a shrub stage to a swamp forest. In such cases the most successful invaders of the tussock association are the willows and *Cornus stolonifera*. The shrubby willows, *Salix interior*, *S. petiolaris*, *S. discolor*, *S. lucida*, *S. bebbiana*, and *S. cordata*, are more aggressive than the tree willows, *S. amygdaloides* and *S. nigra*. All may be found in a single willow thicket bordering the tussock meadow or invading it from the pond side.

The willow stage is usually succeeded by a tree stage characterized by *Fraxinus nigra*, *F. americana*, *Quercus bicolor*, *Ulmus fulva*, and *Acer rubrum*. The shrubs *Alnus* sp., *Ribes grossularia*, *Cornus stolonifera*, *Sambucus canadensis*, and *Xanthoxylum americanum*, and the liana *Psedera quinquefolia* are frequent. The herbaceous layer may consist principally of sedges and grasses if the forest is open. In the shaded areas *Boehmeria cylindrica*, *Pilea pumila*, *Parietaria pennsylvanica*, *Laportea canadensis*, *Eupatorium purpureum*, and

Lycopus americanus are common. In open spaces in these forests tussocks sometimes persist.

In heavily grazed areas the *Eleocharis-Juncus-Carex* complex is generally absent. Bluegrass pasture directly borders the tussock association. The coarser culms of the sedge are apparently unpalatable to livestock, since the grass is eaten down to the bare earth before the sedges are touched. Occasionally the marginal tussocks around ponds are killed by livestock and remain as low, grass covered mounds. This has led to a generally accepted opinion among farmers that tussock meadows result from the trampling of cattle. The best development of the tussock meadow, however, appears in areas where domestic animals are never permitted, especially along railroad rights of way.

Environmental factors within the association

Evaporation

During the summer of 1929 atmometer studies were made in the area south of Cedarburg. Stations were established as follows: (1) in the flat tussock meadow, (2) in the hillside tussock meadow (on an east facing slope), (3) in the *Eleocharis-Juncus-Carex* complex, and (4) in the adjoining grassland. The atmometers were run in triplicate, with one bulb placed at 20 cm., one at 50 cm., and one at 120 cm. above the ground. In every case the third bulb was well above the level of the vegetation. The results are summarized in table III.

It is evident from the data obtained that evaporation above the vegetation varies little in rate over the entire area. The rates recorded are probably higher than the average, owing to the continued drought of that year. In a nearby beech-maple forest at a height of 20 cm. the evaporation rate varied from 6 to 14 cc. per day for the same period. The only effect of the drought on *Carex stricta* that was observed was the folding of the leaves on dry hot days. Long after the grassland had dried up and turned brown, the tussock meadow was still green and luxuriant.

The slight evaporation in the lower levels of the tussock meadow accounts for the persistence of many of the shade loving herbaceous

forms. Supplied with an abundance of moisture for their roots and subject to the greater humidity beneath the sedge cover, they were little affected by the drought. It was to be expected that evaporation near the ground would be influenced by the density and structure of the vegetation. In passing from station 1 to station 4 the increasing evaporation rate in the lower levels is well shown in table III. Correlated with this is the increasing openness of the stand and

TABLE III

AVERAGE DAILY EVAPORATION RATES (IN CC.) AT DIFFERENT LEVELS IN TUSSOCK MEADOWS AND ADJACENT ASSOCIATIONS

STATIONS	HEIGHT OF ATMOMETER BULBS		
	20 CM.	50 CM.	120 CM.
(1) Flat tussock meadow	9.1	21.2	38.1
(2) Hillside tussock meadow	11.3	24.6	39.0
(3) Eleocharis-Juncus-Carex complex	18.2	32.4	42.8
(4) Grassland	36.1	43.4	44.9

the decreasing evidence of stratification. The stratification that is evident in many tussock meadows is due in great part, as YAPP (**15**) has shown for other meadows, to variations in the rate of evaporation at different levels. The obvious conclusion is that evaporation plays little part in determining where a tussock meadow will grow, but that it must be considered a factor which helps determine the floristic composition of the meadow.

SOIL

The soil in these areas is usually a clay or silt loam. The surface layer of 12 to 15 inches is a black compact loam, rich in organic matter. Occasionally it is peaty in nature or shows a fibrous condition as a result of its plant remains. The color is brown or black, depending upon the degree of decomposition. The texture may vary according to the amount of material washed in from the surrounding uplands. The surface layer may be underlain by grayish or fine white sand, or occasionally by a zone of calcareous pebbles. The subsoil is a bluish silty clay which extends downward for several feet.

The surface of these areas is level or it slopes very gradually toward the adjoining drainage courses. Drainage is deficient, the surface water frequently standing for some time in the spring. Areas occurring along stream courses may be subject to overflow. Since the soil of the region is mainly derived from the Niagara limestone, the soils of the tussock meadows are alkaline, owing to the percolation of lime-containing water from the adjacent uplands.

SOIL REACTION.—More than 200 tests were made in the tussock meadows of Washington, Ozaukee, Milwaukee, and Waukesha counties. In every case the soil was found to be alkaline. The pH ranged from 7.2 to 8.1, with an average of 7.4. The lower soil horizons tended to be slightly more alkaline than the surface layer. The water of ponds and small streams showed approximately pH 8. No difference was noted between the soil reaction at the bases of tussocks and that in the tussocks themselves.

Although the soil of tussock meadows in the area studied was consistently alkaline, pH does not appear to be a limiting factor. Several tussocks were dug up and transplanted to the edge of Cedarburg Bog where the water is continuously acid. After two years the sedges were still growing, although subject to the competition of the bog vegetation, particularly *Phragmites communis*, *Carex filiformis*, and *Salix candida*. In the greenhouse *Carex stricta* grows luxuriantly in *Sphagnum* moss. When watered with tap water (from Lake Michigan) the moss showed a pH of 7.02. When watered with distilled water, or with water to which a slight amount of sulphuric acid had been added, the pH varied from 5.6 to 6.8. No differences in growth or color of the plants could be observed throughout this range.

SOIL MOISTURE.—The range of soil moisture in different stands of the tussock association was very great; in individual stands a marked seasonal variation was also observed. Owing to the large amount of organic matter in the spongy upper layer of the soil, the typically poor drainage, and the frequent occurrence of springs, an excess of moisture was present for a considerable part of the season. Based on the dry weight of the soil, the moisture content frequently exceeded 250 per cent. With the lowering of the water table the moisture content slowly dropped until it reached 60 to 80 per cent in late summer. In the subsoil the water content was much lower,

ranging from 20 to 60 per cent. In August, soil moisture in the *Eleocharis-Juncus-Carex* complex ranged from 40 to 60 per cent in the upper 6 inches and slightly less in the subsoil. Soil moisture was never reduced to the hygroscopic coefficient or to a point where it was unavailable for plant growth. Consequently it doubtless is never a direct limiting factor in the persistence of the tussock meadow.

WATER TABLE

A close correlation exists between the presence or absence of tussock meadows in an area and the level of the water table. In the areas of maximum development the water table stands at or near the surface throughout the season. The average depth of the water table in the low hummocky area south of Cedarburg, during the summer of 1929, was 4.8 inches. Rains frequently brought the water table to the surface but it always subsided within a few days. At no time during the entire summer did it fall below 11 inches.

The water table reaches its highest level in April and May as a result of the spring rains. At this time the water in ephemeral ponds may stand at a depth of 18 to 24 inches; in flat or gently sloping areas bordering stream courses it usually stands at the surface; on hillsides, where drainage is better, it stands a few inches below the surface. With the advance of summer it is gradually lowered until the minimum is reached in late August or early September, after which it again slowly rises.

Other workers have recognized the influence of the water level on the character of the vegetation. WARMING (10) states, "In many cases ground-water lies too high for certain plants; in other cases it is so far below that the roots cannot utilize it directly or indirectly; in still other cases it is at such a depth as to be reached by the roots at certain seasons, but not at others." STOUT (9) mentions, "It is universally recognized that the amount of water in a soil and the level at which the ground water stands are important direct factors in determining the character of the plant life which is present." It is possible to estimate with considerable accuracy the depth of the water table in these meadows by considering the season of the year and noting the character of the vegetation. "Islands" in the vegetation almost invariably indicate a difference in water level.

Evidence that the level of the water table has a direct influence on the character of the vegetation is furnished by drainage projects which are being carried out in some places. The placing of tile in a tussock meadow will result in the complete disappearance of the tussocks in a very few years. Measurements of the distance to the water table in such areas near Thiensville, Wisconsin, have shown that the water level is from 2 to 4 feet beneath the surface of the soil. Owners of the land thus drained state that 10 years ago the water stood at the surface for a greater part of the growing season and that well developed tussocks were present at that time.

Records of the water table in a small tussock area on a hillside south of Cedarburg were kept during the summers of 1931 and 1932. Small drainage ditches had been dug in order to make the area suitable for an apple orchard. The average level of the water table dropped approximately 6 inches a year. On September 20, 1933, the water table was 34 inches below the surface. Although the sedge vegetation was still present the area was characterized by a marked invasion of grasses, particularly *Poa pratensis*, and numerous weeds, including a few specimens of *Verbascum thapsus* and a great many *Solidago* spp. The dried stems of *Linaria canadensis* and *Prunella vulgaris* were also abundant. It is obvious that drainage is rapidly causing the disappearance of *Carex stricta* in this place.

Biotic factors

Grazing

The effect of grazing upon the tussock vegetation is noticeable only when the number of livestock present greatly exceeds the capacity of the pasture. Most of the better pasture lands are of the bluegrass type, tussock associations occasionally being present as local areas in the pasture. Pure tussock meadows are seldom used for grazing.

Most of the grazing is done by cattle and horses. Ordinarily they graze the tussocks only when the grass has been depleted. If the pasture is overstocked the complete disappearance of the tussocks may result. Around ponds the grass covered mounds, representing former tussocks, may be observed as a zone bordering those which

still remain. Where grazing is not intensive the tussocks may remain for a long time. Bluegrass invades the spaces between them and along with the grazing prevents their vegetative propagation by means of rhizomes. Under these conditions various weeds characteristically grow in the protection of the tussock crown.

Intensive grazing generally results in greater exposure of the soil and consequently in a decrease of moisture and a lowering of the

FIG. 8.—Winter aspect of tussocks with burned-over area at left; water table near surface in summer.

water table. Succession toward a bluegrass pasture is thus hastened. Succession toward the shrub and forest stages does not take place under the influence of grazing.

FIRE

Farmers commonly burn these areas each year after the vegetation has become dry (fig. 8). The opinion prevails that burning will cause the disappearance of the tussocks or improve the grazing qualities of the pasture. It does neither. Burning results in the removal of the dried leaves and culms which serve to fill in the spaces between the tussocks. The roots and rhizomes are not harmed to any great extent by fire. Burning, however, does permit an increased invasion of weeds into the area and drives out or kills the small game animals that frequent the meadow. It is a practice which might well be discontinued.

Rodents

The destruction of tussocks is sometimes hastened by rabbits and mice. When food is scarce these animals sometimes eat the exposed roots and rhizomes just beneath the tuft of dead leaves. In the region of the cut ends of these organs a constriction in the pedestal occurs. The soil surrounding the roots remaining nearest the surface may crumble as a result of frost action or be washed away by rain. Renewed attacks of the rodents may so weaken the pedestal that it topples of its own weight. The destruction of tussocks by this means, however, is negligible.

Discussion

The conditions of the habitat occupied by the tussock sedge lie within the limits of tolerance of many other species; consequently the explanation of the dominance of *Carex stricta* in the tussock association must be sought primarily in the characteristics of the plant itself. Among the characteristics of a dominant Weaver and Clements (12) include size, abundance, and duration. *Carex stricta* has all of these.

The chief competitors of the tussock sedge are species of similar form, the grasses and other sedges. Few of these are taller than *Carex stricta* and all lack the additional advantage in height contributed by a pedestal. In early spring when growth begins, the height of the pedestal represents an advantage which, under favorable conditions, is generally maintained. Thus species which grow concurrently with the tussock sedge are at a disadvantage from the beginning.

The abundance of culms is apparent even at a glance. Statistical studies show that *Carex stricta*, in areas of maximum development, may constitute almost 100 per cent of the vegetation. Abundant tillering takes place and new buds and culms may be produced throughout the summer.

The perennial nature of the tussocks and the considerable age to which they attain are factors which help to bring about their permanent occupancy of an area. The mass of dead leaves and the matted roots and rhizomes which are added to the soil each year tend to create a fibrous peat which is favorable to the continued growth of the sedge.

The cespitose habit contributes to the dominance of *Carex stricta* in still another way. It constitutes an admirable adaptation to a fluctuating water level. During that portion of the growing season when the water is well above the surface of the ground, the leaves and stems are seldom if ever submerged. Aeration of roots and rhizomes presents no problem, owing to their well developed aerenchyma. With the lowering of the water table and the consequent decrease in moisture supply in the region of the fibrous roots, the soil roots in the zone of saturation supply the necessary moisture. The demand for moisture during times of stress is lessened to some extent by the folding of the leaves.

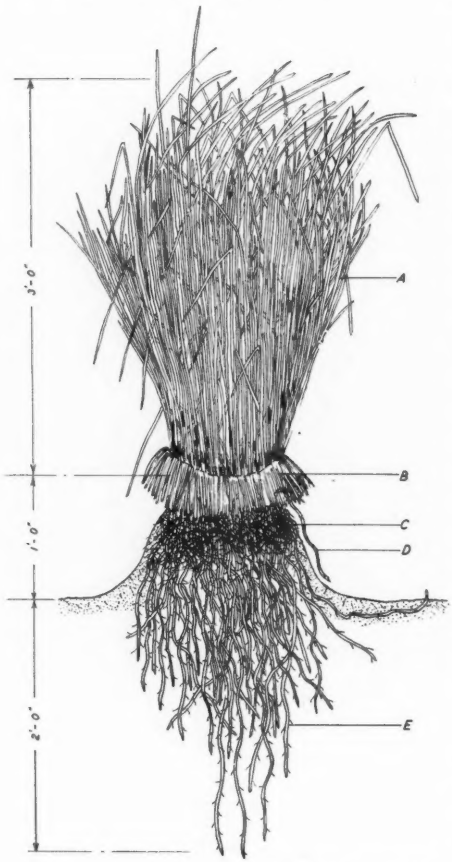


FIG. 9.—Diagrammatic sketch of a *Carex stricta* tussock: *A*, leaves of current growing season; *B*, dead leaves of previous season; *C*, fibrous roots in earthy pedestal; *D*, rhizome; *E*, wirelike soil roots.

The factors which appear most to limit the growth of the tussock sedge are deficiency of water, soil type, shade, and grazing. A water depth greater than 18–24 inches in ponds is usually not conducive to the growth of tussocks. Likewise, *Carex stricta* is seldom present in areas where the water table falls more than 18–24 inches below the surface during the growing season. The rapid disappearance of

the association following drainage is evidence in support of this view. The moisture content of the soil may also be correlated with the average depth of the water table. In well developed tussock meadows the moisture content of the soil seldom falls below 60 per cent during the growing season. In adjacent associations where *Carex stricta* is absent it may fall as low as 15 per cent during periods of drought.

Soils low in organic matter never support tussock vegetation. In fact it appears that the soil must be somewhat peaty before the tussock sedge can even invade an area. Again, a water relation may exist, since peaty soils are capable of holding a high percentage of moisture. On the other hand the tussocks do not occur on sandy substrates, especially where the drainage is good and the moisture content falls rapidly with the advance of the dry season.

The effect of shade is especially apparent when the association is being invaded by shrubs and trees. Permanent quadrats, which were observed for a period of five years under medium sized trees of *Salix amygdaloides*, *S. nigra*, and *Ulmus americana* which had become established in an otherwise pure tussock association, show the disappearance of certain of the tussocks and a gradual invasion of herbaceous dicotyledonous forms, both in the spaces between the pedestals and in the crowns of the tussocks themselves. Quadrats in areas being invaded by shrubby willows show an even faster rate of disappearance.

Among the factors of the environment which do not appear to be limiting are evaporation and soil reaction. Although the evaporation rate varies in the various levels of the association and determines to an extent the nature of the secondary vegetation, it is little different at the upper level of the tussock meadow from the rate in adjacent associations. It does not appear to be a factor in succession. Similarly the hydrogen ion concentration of the soil in the different soil horizons and in the associations preceding and following the tussock association appears to remain fairly constant. Variations that occur are not large enough to be significant.

The omission of the tussock association in certain successions seems worthy of note. This is especially true of lakes with sandy shores and the margins of quaking bogs. In many of these areas the

proper soil is lacking although the water content may be high. Although *Carex stricta* has been grown in sand in the greenhouse, with difficulty, its ability to compete with sand-loving species in nature is doubtful. It is probable that this species has been excluded from the margins of many lakes as a result of its inability to migrate. Although the seeds are easily carried they have not been observed to germinate in nature, and all attempts to germinate them in the laboratory have failed. In addition, a surprisingly small number of fruiting plants have been found in the region studied.

The rapid succession that takes place around many lakes is also a factor which serves to exclude the slowly developing tussock sedge. In many instances the shrub stages are found at the very margins of lakes. In such cases light enters immediately as a limiting factor. Similarly the shrub stages in bogs may encroach rapidly upon the sedge vegetation, working destruction on species that depend more upon permanent establishment than upon migratory ability for their existence. The unstable substratum of the floating mat is also a poor foundation for a plant which needs firm anchorage for its pedestals. The effect of physiological conditions in bogs upon *Carex stricta* cannot be stated. Tussocks transplanted to bogs, however, have grown with a moderate degree of success for a period of two years.

Summary

1. The vegetation and environmental conditions of tussock meadows in southeastern Wisconsin were studied during the summers of 1929, 1932, and 1933.

2. Tussock meadows are developed along stream and pond margins, on springy hillsides, and in drained morainic depressions. They are found on soils showing a high percentage of organic matter.

3. Tussock meadows are dominated by a single species, *Carex stricta*, which produces tussocks that consist of a tuft of leaves and culms at the summit of a pedestal composed of roots, rhizomes, soil, and vegetable débris.

4. New tussocks are initiated by means of rhizomes which grow horizontally from the bases of old tussocks.

5. Individual tussocks may attain a height of 2 to 4 feet and a diameter of 8 to 30 inches. They may persist in an area for many years.

6. The leaves of *Carex stricta* show certain xeromorphic structures which indicate an adaptation to the excessive evaporation rates to which they are occasionally subjected. The roots and rhizomes show hydromorphic structures which indicate an adaptation to extremely moist conditions.

7. The tussock habit enables *Carex stricta* to persist in areas where the water level shows a marked seasonal fluctuation.

8. The tussock association is almost exclusively herbaceous. The most important secondary species are grasses and sedges. Dicotyledonous herbs may dominate in appearance but not in numbers at certain seasons.

9. *Carex stricta* furnishes marked competition for herbaceous species from the standpoint of both aerial and subterranean organs.

10. Progressive decrease in soil moisture and lowering of the water table lead to invasion by species of lower moisture requirements and the ultimate disappearance of the tussock association.

11. In succession, the tussock association is usually preceded by a reed-swamp stage and followed by a sedge-rush stage. The association may be invaded by willows which are eventually succeeded by a swamp forest.

12. Evaporation, annual fires, and soil reaction are not significant factors in succession.

13. Overgrazing may result in the disappearance of the tussock association and the appearance of grassland in its place.

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GROWTH STUDIES IN RELATION TO ULTRAVIOLET RADIATION[1]

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(WITH THIRTEEN FIGURES)

Introduction

The effect of ultraviolet radiation upon physiological processes has been investigated by animal physiologists and the treatment has found manifold applications in therapeutics, but our knowledge with respect to its influence upon plant processes is yet meager. The investigations so far reported appear to be more or less of a qualitative nature, dealing with either the indirect effect of these rays when cut off from sunlight or their direct influence when supplied in extra doses, notwithstanding the fact that the after-effects in the two cases could wholly be attributed to the same cause. Thus BONNIER and MANGIN (2), using appropriate filters, discovered the occurrence of photosynthesis under the influence of these rays, while URSPRUNG (10) failed to find even a trace of starch on irradiating leaves with a quartz mercury apparatus. SCHANZ (6) and POPP (5) have emphasized the detrimental effect of these rays upon plant growth, but TINCKER (9) stresses their utility in giving better stocky growth and higher yield. A lack of exact knowledge regarding the source of radiation, the intensity of the incident rays, the duration of exposures, and the precise stage in the ontogeny when such treatments will have maximum possibilities of growth and yield adds to the difficulty of assessing at its real value the efficiency of such rays in plant growth.

The present investigations were taken up during the years 1931–34. Pure strains of *Triticum vulgare* (var. Pusa 4), *Linum usitatissimum* (var. 1150 S), *Gossypium hirsutum* (var. New Amer. Upland), *Crotalaria juncea* (var. local), *Oryza sativa* (var. Jilhore), and *Nicotiana tabacum* (var. Puca 177), raised in well drained, large sized

[1] Contribution from the Institute of Agricultural Research, Benares Hindu University, India.

containers under optimum conditions of nutrition, were subjected to ultraviolet rays of constant intensity from a 220-volt quartz-inclosed unscreened mercury vapor lamp held in a standard parabolic reflector, operated on 143 watts (130 volts, 1.1 amperes).

At successive intervals after treatment with these rays, the required number of plants from each of the series were selected in the morning hours and the fresh weight, leaf area, structural abnormalities, and other characteristics recorded. The material was finally incubated at 100°C. to yield the dry weight. A part of the material was prepared after LINK and TOTTINGHAM (3) for the estimation of acid-soluble carbohydrates by Pavy's method. Total nitrogen was determined by the modified Kjeldahl's method (4). In each case the after-effects of irradiation were studied in terms of morphological variabilities, growth activity, and the carbohydrate/nitrogen drifts at successive stages of the life-cycle.

Experimental findings

To study the effect of ultraviolet rays in their various aspects, a considerable number of experimental plants, of as nearly uniform size and weight as possible, were exposed to such rays for varying durations administered at shorter or longer intervals as follows:

A. AT INTERVALS SHORTER THAN A FORTNIGHT:
   (1) 5 minutes daily
   (2) 15 minutes on alternate days
   (3) 30 minutes on alternate days
   (4) 5 minutes weekly
   (5) 15 minutes weekly
   (6) 30 minutes weekly

B. AT INTERVALS LONGER THAN A FORTNIGHT:
   (1) 15 minutes fortnightly
   (2) 30 minutes fortnightly
   (3) 15 minutes twice in the life-cycle
   (4) 15 minutes once in the life-cycle

The experimental plants were exposed for the first time on the twenty-second day after germination when they were more or less

independent organisms, while subsequent irradiations depended to a large extent upon the particular dose the effect of which was under study.

A. Experiments on irradiation of plants at intervals shorter than a fortnight

Ten days after treatment, plants belonging to different sets begin to exhibit marked changes in their morphological features, the variations becoming more characteristic with the advance in age of the plants. Plants irradiated for 5, 15, and 30 minutes (except in case of *Linum* treated for 15 minutes on alternate days) fail to develop normally. Morphological differences in the general vigor, height, leaf area, stem development, and grain formation soon become apparent. The variations, although not very clearly defined in all the cases, when compared with the control, are more pronounced when either the time interval between two successive exposures is as short as only 24 hours or when the duration of each exposure is half an hour. Plants belonging to each of these sets are smaller in comparison with the control (table I) and have few or no branches. Leaves do not develop to the normal size and assume a pale yellowish green color within two to three weeks after the first treatment, showing a tendency to die prematurely. Attenuation is another outstanding feature in the unfavorable effects of these short-intervaled irradiations. When *Triticum* receives 5 minutes' daily irradiation, culm elongation is greatly inhibited resulting in the stunted growth of plants with reduced tillering, short narrow leaves, and small poorly developed heads (fig. 1). The variabilities are equally interesting in those sets where the duration of each exposure is as long as 30 minutes, even though the time interval is of a week's length. In addition to these variations in the general features, shedding of leaves and flowers is also common under this treatment. The resulting bolls in *Gossypium*, pods in *Crotalaria*, and ears in *Oryza* are fewer in number and reduced in size, having few small shriveled seeds or none at all. In extreme cases only a naked rachis remains. The results obtained for *Crotalaria* are rather characteristic (fig. 2). Longer exposures are thus more damaging to the yield of plants than shorter ones supplied very frequently.

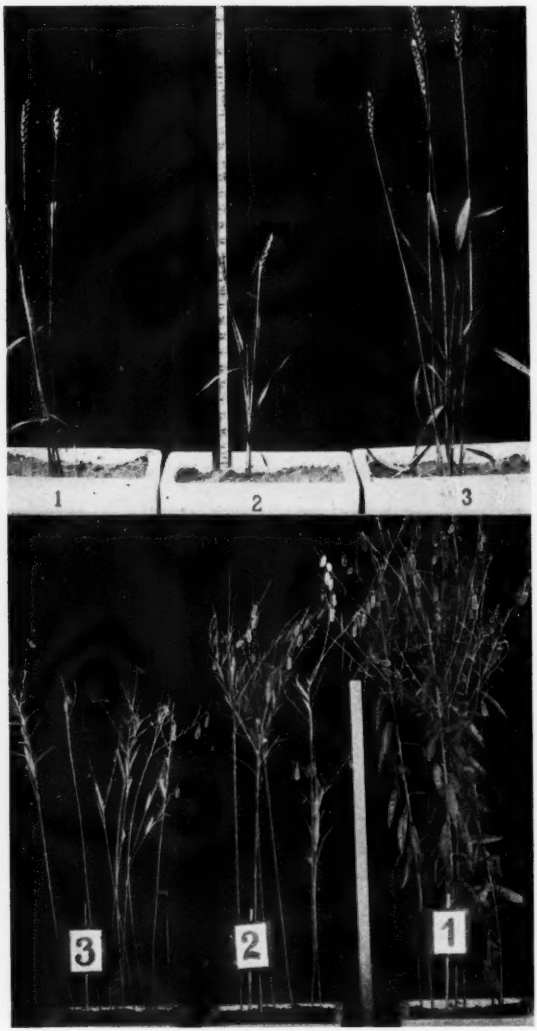
TABLE I

INFLUENCE OF ULTRAVIOLET RADIATION UPON PLANT GROWTH

Item	Control	Irradiations at shorter intervals						Irradiations at longer intervals			
		Daily	Alternate days		Weekly			Fortnightly		Twice in life-cycle	Once in life-cycle
		5 min.	15 min.	30 min.	5 min.	15 min.	30 min.	15 min.	30 min.	15 min.	15 min.
	Triticum vulgare										
Av. height per plant in cm.	55.00±0.478	35.00±0.395	51.50±0.541	46.00±0.520	.....	52.00±0.590	.....	67.50±0.872	53.00±0.653	.....	.....
Av. number of tillers per plant	3.40±0.121	2.10±0.140	2.70±0.016	2.30±0.035	.....	3.00±0.142	.....	4.60±0.179	3.20±0.050	.....	.....
Final dry weight per plant in gm.	6.97±0.174	3.03±0.082	6.12±0.142	5.10±0.105	.....	6.75±0.165	.....	8.49±0.178	6.84±0.134	.....	.....
Av. length of heads in cm.	8.50±0.101	6.07±0.080	6.50±0.091	6.00±0.072	.....	7.80±0.041	.....	10.30±0.135	8.30±0.220	.....	.....
Av. yield of grain per plant in gm.	3.52±0.065	1.56±0.024	2.36±0.046	2.16±0.051	.....	2.49±0.035	.....	3.76±0.072	3.16±0.055	.....	.....
Absolute weight of 100 grains in gm.	4.35±0.111	1.74±0.030	1.94±0.016	1.87±0.043	.....	3.15±0.061	.....	4.78±0.130	4.12±0.101	.....	.....
Volume weight of grains per cc.	0.66±0.071	0.21±0.005	0.28±0.008	0.22±0.012	.....	0.54±0.041	.....	0.68±0.085	0.61±0.054	.....	.....
Period of blossoming in days	50	36	43	43	.....	50	.....	50	50	.....	.....
Duration of floral development in days	21	14	14	14	.....	14	.....	14	14	.....	.....
Fruiting stage in days	71	50	57	57	.....	64	.....	64	64	.....	.....
Harvesting period in days	106	78	92	85	.....	99	.....	106	99	.....	.....
	Linum usitatissimum										
Av. height per plant in cm.	32.50±0.540	25.00±0.327	34.00±0.463	29.50±0.395	.....	30.50±0.452	.....	46.00±0.475	50.00±0.680	.....	.....
Final dry weight per plant in gm.	2.93±0.055	1.40±0.023	3.64±0.048	2.33±0.042	.....	2.33±0.042	.....	6.57±0.160	7.64±0.182	.....	.....
Av. yield of grain per plant in gm.	1.94±0.032	0.30±0.008	2.02±0.051	1.08±0.020	.....	1.10±0.015	.....	3.60±0.062	3.95±0.081	.....	.....
Period of blossoming in days	50	36	36	36	.....	42	.....	50	50	.....	.....
Duration of floral development in days	28	14	14	14	.....	14	.....	14	14	.....	.....
Fruiting stage in days	78	50	50	50	.....	56	.....	64	64	.....	.....
Harvesting period in days	106	78	92	85	.....	99	.....	106	99	.....	.....

TABLE I—*Continued*

Item	Control	Irradiations at shorter intervals						Irradiations at longer intervals			
		Daily	Alternate days		Weekly			Fortnightly		Twice in life-cycle	Once in life-cycle
		5 min.	15 min.	30 min.	5 min.	15 min.	30 min.	15 min.	30 min.	15 min.	15 min.
Gossypium hirsutum											
Av. height per plant in cm.	77.50±0.840				72.50±0.782	56.50±0.620	45.00±0.572				
Final dry weight per plant in gm.	105.00±0.820				87.60±0.542	51.90±0.560	30.60±0.491				
Av. number of bolls per plant	10.50±0.210				8.20±0.151	7.00±0.220	5.10±0.052				
Av. weight of seed-cotton per plant in gm.	15.20±0.248				11.52±0.127	7.12±0.102	4.30±0.082				
Av. weight of cotton-seed pe- plant in gm.	10.40±0.230				8.06±0.105	4.56±0.087	3.40±0.040				
Period of blossoming in days	71				66	66	64				
Duration of floral development in days	28				26	26	26				
Fruiting stage in days	99				92	92	90				
Harvesting period in days	127				120	120	120				
Crotalaria juncea											
Av. height per plant in cm.	106.20±0.780				87.50±0.560	75.00±0.450	68.70±0.495			110.50±0.630	108.00±0.845
Final dry weight per plant in gm.	6.20±0.155				4.44±0.159	4.00±0.105	2.16±0.032			9.53±0.225	8.35±0.090
Av. number of pods per plant	10.80±0.065				3.30±0.170	2.80±0.135	2.00±0.083			15.70±0.213	11.80±0.110
Av. yield of grain per plant in gm.	1.84±0.015				1.23±0.012	1.01±0.021	0.61±0.004			1.98±0.035	1.92±0.030
Period of blossoming in days	43				42	40	40			43	43
Duration of floral development in days	21				17	17	17			14	14
Fruiting stage in days	64				59	57	57			57	57
Harvesting period in days	99				93	92	92			98	98
Oryza sativa											
Av. height per plant in cm.	37.50±0.556				30.00±0.482	25.50±0.491	22.50±0.350				
Final dry weight per plant in gm.	3.91±0.072				2.23±0.081	1.69±0.045	0.52±0.013				
Av. yield of grain per plant in gm.	0.97±0.031				0.80±0.030	0.51±0.013	0.20±0.005				
Period of blossoming in days	71				66	64	64				
Duration of floral development in days	16				14	14	14				
Fruiting stage in days	87				80	78	78				
Harvesting period in days	106				101	99	99				



Figs. 1, 2.—Fig. 1 (above), fully developed plants of *Triticum:* (1) control, (2) 5 minutes every day, and (3) 15 minutes fortnightly. Fig. 2 (below), fully developed plants of *Crotalaria:* (1) control, (2) 5 minutes weekly, (3) 30 minutes weekly.

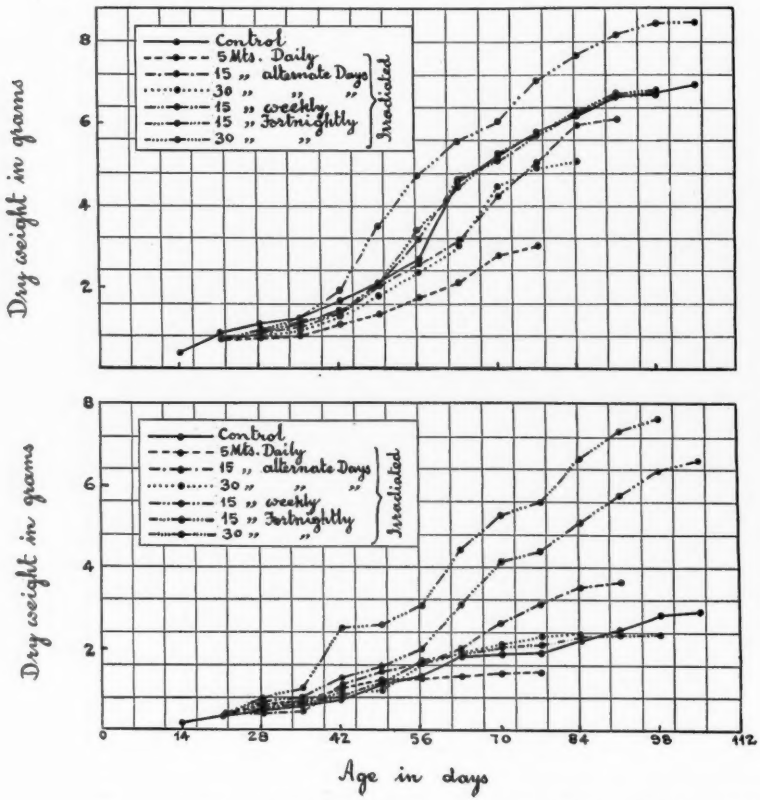
There is a tendency for these short-intervaled irradiations to inhibit vegetative growth and force early flowering and maturity, associated with reduced yield of grain (table I). Treatments administered more frequently (daily) or given for longer durations (30 minutes per exposure) induce such changes to a greater extent. Average weights of the oven-dried entire plants taken at successive stages further indicate that, with the exception of *Linum*, all other plants thus treated show a lack of dry matter accumulation at all stages following treatment (fig. 3, table I). *Linum* exhibits an increase in the dry weight for several successive weeks during the course of treatment (fig. 4) and is thus more resistant to such doses.

B. Experiments on irradiations of plants at intervals longer than a fortnight

When the time interval between two successive exposures is increased to a fortnight or more, even 30 minutes' dose induces rapid development and increased growth in *Linum*. In *Triticum* 15 minutes' dose supplied at similar intervals induces a highly vegetative growth with increased tillering and large greenish leaves in contrast to control plants (fig. 1). Such irradiations also produce longer heads, apparently as a result of abnormal elongation of the internodes of the rachis. The average weight of grains per plant after such irradiations exceeds that of the control plants. The grains produced are also superior in quality, in so far as their absolute and volume weights are concerned. The plants are unusually tall (table I).

Of the plants thus studied, *Crotalaria* is found to be exceedingly sensitive to the action of these rays, for when irradiated even once in the age-cycle, appreciable increments in the size of both the foliage and the floral organs are noticed. Increased height with high grain yield is observed in those plants which are exposed twice in the life-cycle, once as usual on the twenty-second day after germination and subsequently after a fortnight; that is, a few days preceding flowering (table I). This indicates that a single irradiation in *Crotalaria* is by no means sufficient to ensure maximum possibilities of growth and yield. *Nicotiana* is almost equally sensitive to such rays. In most of the plants thus tested the periods of flowering and maturity

are not markedly affected, while the dry matter accumulation and yield are considerably increased (tables I, II). Doses of 30 minutes at intervals of a fortnight are slightly harmful to the growth of *Triticum*.



Figs. 3, 4.—Fig. 3 (above), time-weight curves for *Triticum* showing relative increment in dry matter accumulation of entire plant after irradiation. Fig. 4 (below), time-weight curves for *Linum* showing relative increment in dry matter accumulation of entire plant after irradiation.

SEED TREATMENT.—Seeds of *Nicotiana* after soaking in 1 per cent sodium chloride for an hour were dried at laboratory temperature and exposed for 5, 10, and 20 minutes respectively to ultraviolet rays.

Seeds treated for 5 and 10 minutes are found to germinate and grow more vigorously and in larger numbers than either the control or those treated for 20 minutes, which in their turn exhibit poorer germination even than the untreated ones (table II). The subsequent growth and other morphological characteristics also undergo marked changes in response to variations introduced during the

Fig. 5.—Fully developed plants of seed-treated *Nicotiana:* (1) control, (2) 5 minutes, (3) 10 minutes, (4) 20 minutes.

early seedling stage in the various experimental sets. Seeds treated for 5 and 10 minutes respectively present taller plants greatly surpassing the controls (fig. 5), with bigger leaves and a more extensively developed root system. Of these, a dose of 5 minutes' duration has only a transitory effect, giving increased leaf yield (table II). Exposure of 10 minutes' duration, on the other hand, accelerates equally the development of foliage and inflorescence, bringing considerable increase in both the leaf and the grain yield. Blossoming and maturity are not much affected by such irradiations. Plants

belonging to 20 minutes' seed-treated sets on the other hand are forced into reproductive stage so rapidly that there is an insufficient development of leaves and stems (fig. 5). They matured one week earlier than the control plants and gave poor yield of both leaf and grain (table II).

ABSOLUTE GROWTH AND GROWTH RATE.—Control time-weight curves for the crop plants under investigation are more or less of a sigmoid type (figs. 3, 4), a detailed description of which has already appeared (7). Comparing these curves with those obtained for irradiated plants, a striking similarity in their forms is noticed which indicates that the dynamics of growth is not fundamentally affected in response to ultraviolet radiation. The consistent variation in their level under identical conditions of environment, however, points to the change being conditioned by a variation in the internal factors of the plant as a result of irradiation, leading to a greater accumulation of dry matter in some cases and lesser in others.

The relative growth rate curves belonging to the experimental series of plants depict a greater slope than their respective checks in the week following the treatment, indicating that the immediate after-effect of these rays is to retard growth (figs. 6–9). The maximum growth point in each case is attained on the approach of flowering, and hence irradiations at intervals shorter than a fortnight (except in *Linum*) are associated with earlier growth maxima in contrast to check plants. A stricter comparison of these curves reaffirms that the series of plants producing higher yield are essentially those preceded by higher growth maxima and vice versa.

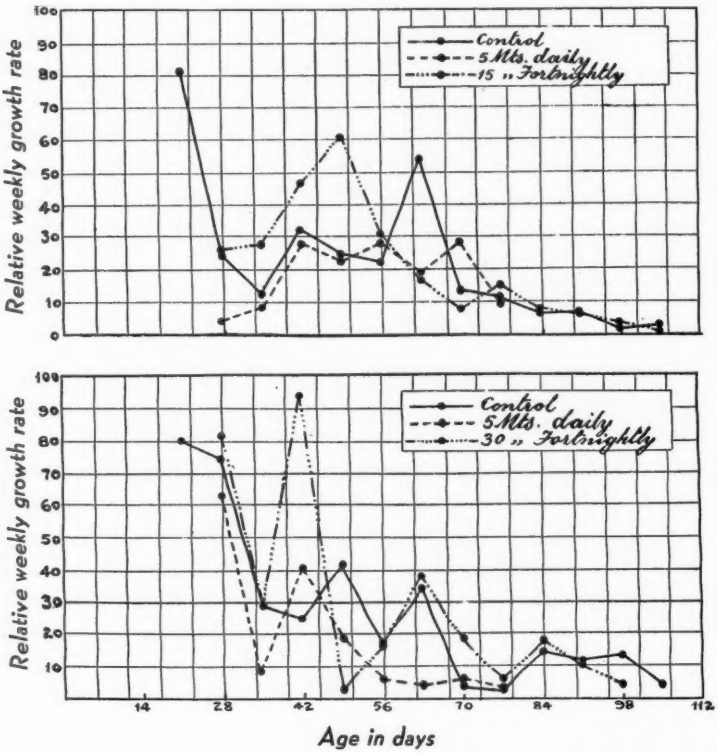
CARBOHYDRATE/NITROGEN DRIFTS.—To obtain some indication regarding the developmental variations, and also to reveal the physiological balance in plants on exposure to ultraviolet rays, carbohydrate/nitrogen determinations were made. Figure 10 shows that the percentage of carbohydrates at similar stages in the control and seed-treated series of *Nicotiana* varies from set to set, although the nature of the curves remains identical inasmuch as they are all characterized by a preflowering maximum. Five and 10 minutes' seed-treated plants are associated with higher carbohydrate percentages than the check, while those whose seeds received treatment for 20 minutes show lower percentages. As far as the nitrogen is concerned,

TABLE II

RELATIVE EFFECTS OF SEED AND PLANT IRRADIATION IN NICOTIANA TABACUM

ITEM	CONTROL	SEED TREATMENT			PLANT TREATMENT ONCE IN LIFE-CYCLE
		5 MINUTES	10 MINUTES	20 MINUTES	
Percentage capacity for germination	70	79	91	46	.....
Speed of germination in days	20	18	15	25	.....
Average height per plant in cm	68.75 ±0.491	81.25 ±0.640	87.50 ±0.620	62.50 ±0.385	84.50 ±0.530
Final dry weight per plant in gm	26.92 ±0.206	31.52 ±0.357	39.10 ±0.239	25.70 ±0.255	36.28 ±0.242
Average leaf yield per plant in gm	6.45 ±0.110	8.10 ±0.184	11.50 ±0.138	5.20 ±0.120	10.90 ±0.143
Average grain yield per plant in gm	0.67 ±0.012	0.62 ±0.013	1.88 ±0.020	0.60 ±0.010	1.45 ±0.045
Absolute weight of 100 grains in gm	0.0035±0.0001	0.004±0.0005	0.005±0.0002	0.003±0.0005	0.004±0.0006
Volume weight of yield grains per cc	0.367 ±0.003	0.368±0.007	0.423±0.011	0.352±0.015	0.400±0.042
Period of blossoming in days	94	94	94	89	94
Duration of floral development in days	14	12	12	12	12
Fruiting stage in days	108	106	104	101	106
Harvesting period in days	129	127	127	122	127

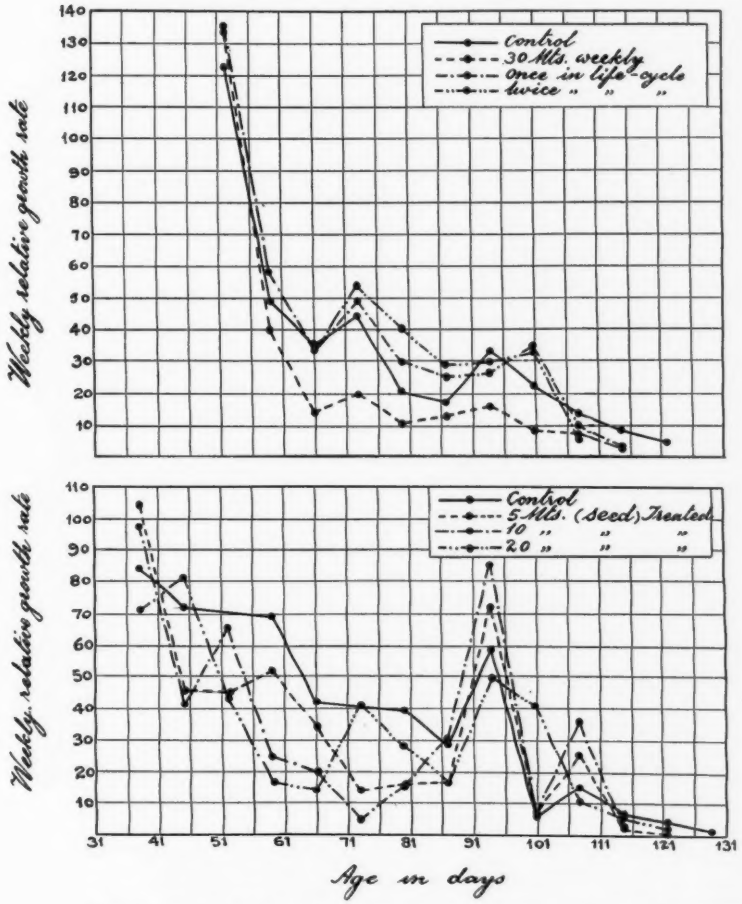
there is a progressive decrease in its percentage with increase in the duration of exposures (fig. 11). Unlike carbohydrates, therefore, in none of the irradiated sets does nitrogen percentage increase beyond



Figs. 6, 7.—Fig. 6 (above), relative growth rate curves of control, 5 minutes daily, and 15 minutes fortnightly treated plants of *Triticum*. Fig. 7 (below), relative growth rate curves of control, 5 minutes daily, and 30 minutes fortnightly treated plants of *Linum*.

that of the check. The carbohydrate/nitrogen ratio curves (fig. 12), however, resemble those for carbohydrates belonging to similar series. Plants of 10 minutes' seed-treated series giving high grain yield are characterized by the highest carbohydrate/nitrogen ratio,

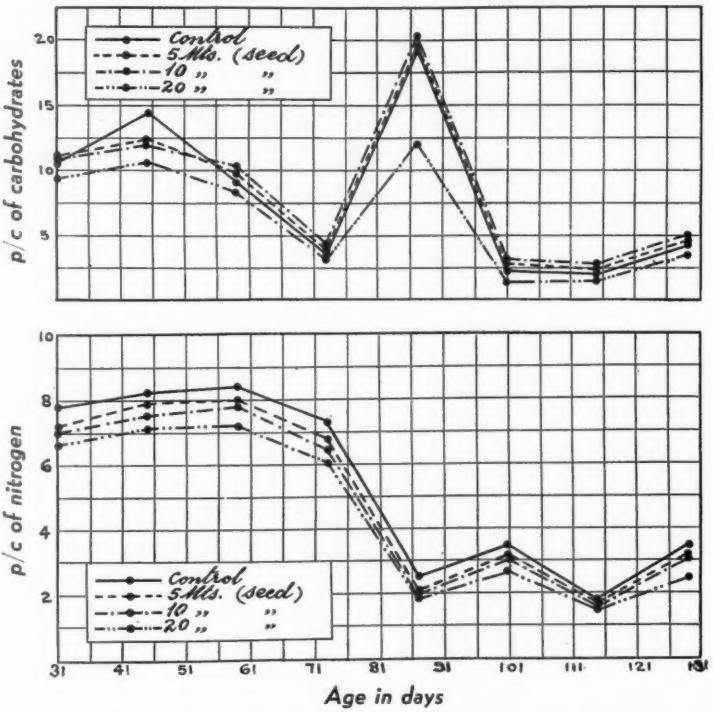
while those of the 20 minutes' series where the development of plants is inadequate are associated with very low ratio.



Figs. 8, 9.—Fig. 8 (above), relative growth rate curves of control, 30 minutes weekly, once in life-cycle, and twice in life-cycle treatments in *Crotalaria*. Fig. 9 (below), relative growth rate curves of seed-treated *Nicotiana*.

NET ASSIMILATION RATE.—The values of net assimilation rate calculated from dry weight and leaf area data are taken into account to

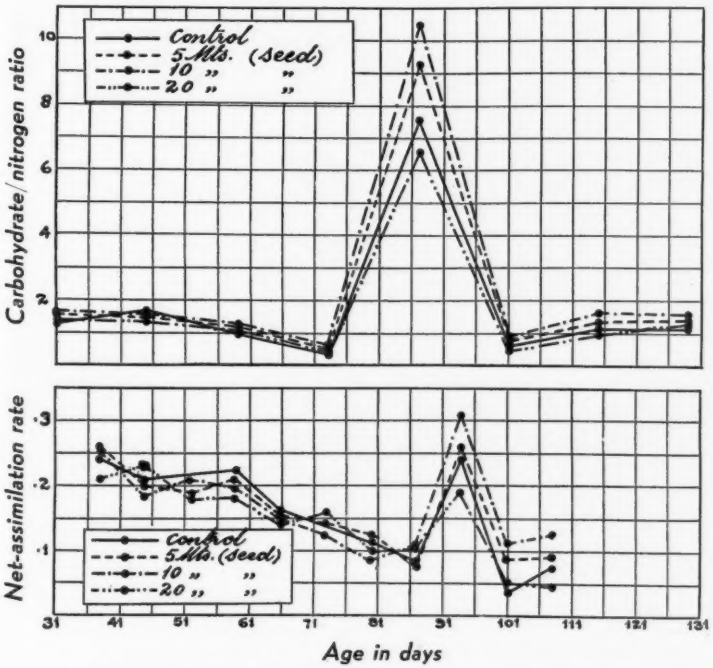
explain the induced variations brought about by ultraviolet rays in the carbohydrate and nitrogen accumulations. Figure 13 indicates that the assimilation rates also undergo marked changes like those obtained in the carbohydrates. Five and 10 minutes' seed-treated



Figs. 10, 11.—Fig. 10 (above), carbohydrate percentages in seed-treated plants of *Nicotiana*. Fig. 11 (below), nitrogen percentages in seed-treated plants of *Nicotiana*.

plants, therefore, depict higher while those developing from seeds treated for 20 minutes depict lower rates, in comparison with the control. The relative growth rate curves for the seed-treated and control series show more or less similar features, inasmuch as the maxima in the two cases fall at the same stage (figs. 9, 13). High assimilation rates are therefore correlated with high carbohydrate accumulation and high growth rates, and vice versa.

Results obtained in the present investigation thus show that variations in the growth and yield of plants on exposure to ultraviolet radiation are associated with fluctuations in the assimilation rate and consequent changes in the nutrient level of the plants.



Figs. 12, 13.—Fig. 12 (above), carbohydrate/nitrogen ratio in seed-treated *Nicotiana*. Fig. 13 (below), net assimilation rate of seed-treated plants of *Nicotiana*.

In the light of these experimental findings it is to be admitted that certain doses of ultraviolet rays are beneficial in increasing the growth and yield of plants. BENEDICT (1) has recently shown that ultraviolet rays of 2900–3100 Å are useful to the growth of plants while still shorter waves produce harmful effects. But since such rays are rarely transmitted through the epidermis, as shown by BENEDICT, the extent of resulting harm due to these rays may be considered to be markedly less. In the present case, however, the

range of wave length is decidedly greater since no filters were used, but the plants raised under natural conditions of environment are subjected to such rays only occasionally, and hence in some cases increase in growth and yield takes place. The differential behavior in the growth response of different crop plants is explained on their specific ultraviolet requirement. Like x-radiation (8), therefore, ultraviolet rays also are useful when administered in suitable doses in initiating profitable crop production.

Summary and conclusions

1. The present investigations aimed at a quantitative analysis of the effect of ultraviolet radiation, administered in varying doses, frequently or occasionally, upon the growth and yield of certain crop plants. The definable after-effects consequential to such treatments are estimated in terms of morphological changes, growth behavior, the drifts in the carbohydrate and nitrogen percentages, and the net assimilation rate at successive stages of growth during the entire life-cycle of the crops.

2. Exposure of plants to ultraviolet rays for a period of 5 minutes or more at intervals shorter than a fortnight is detrimental to their growth and yield. The more frequent the exposures or the longer the duration of each exposure, the greater is the harm incurred. Longer exposures at one time are more damaging to the yield of some plants than shorter ones supplied frequently. Occasional treatments of 15 minutes' duration are productive of good growth.

3. Seed treatment for 10 minutes or less accelerates germination and induces luxuriant growth in plants while longer treatments of 20 minutes' duration retard both germination and development.

4. In all cases where early flowering and maturity are the result of ultraviolet exposures, the plants are characterized by stunted growth and reduced yield; whereas under treatments giving better growth and yield as compared with the control, the normal period of vegetative and reproductive growth remains more or less unchanged.

5. The yield of plants subjected to ultraviolet rays is proportional to the magnitude of the time-weight curves as well as to the maximum height of the growth rate curves.

6. Variations in the growth and yield of crops on exposure to ultraviolet rays are explained on the basis of fluctuations in the net assimilation rate and the carbohydrate/nitrogen ratio. The response to these rays appears to be different in different plants.

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EFFECTS OF BARIUM SALTS UPON ASPERGILLUS NIGER AND THEIR BEARING UPON THE SULPHUR AND ZINC METABOLISM OF THE FUNGUS IN AN OPTIMUM SOLUTION

ROBERT A. STEINBERG

During the course of investigations on the nutrition of *Aspergillus niger* the observation was made that, although as found by JAVILLIER (**2**), the addition of small quantities of barium salt to the nutrient solution was without effect upon the growth of the fungus, larger amounts resulted in cultures identical in most respects with the minus iron and minus zinc cultures (**4**). Since the results obtained bore directly upon the interpretation of the influence of iron and zinc on the development of the fungus, additional information was sought to clarify their relation to the heavy metal and sulphur nutrition of the organism.

The nutrient solution employed in these investigations contained 1000 cc. water redistilled through pyrex glass, 50 gm. of sucrose, 2 gm. of ammonium nitrate, 0.48 gm. of dipotassium phosphate, and 0.62 gm. of magnesium nitrate ($Mg(NO_3)_2 . 6H_2O$). To this solution also were added 0.15, 0.12, 0.03, and 0.02 mg. per liter of iron, zinc, copper, and manganese as sulphate, respectively. The solution contained about 1 mg. of sulphur per liter. Its pH was 5.64. A solution having a pH of 4.16 was employed for the barium precipitation experiments. It was obtained by substitution of 0.75 gm. of KH_2PO_4 for the K_2HPO_4 of the preceding formula.

Table I indicates the effects of the use of sulphur in various forms upon the growth and development of the fungus. The data are average values for duplicate cultures in 200 cc. pyrex Erlenmeyer flasks containing 50 cc. of nutrient and grown at 34.7°–35.0° C. for 6 days.

Increases in growth followed upon addition to the nutrient solution of sulphur as sulphuric acid, sodium sulphide, and sodium sulphate. The last appeared most effective in supplying the needs

of the organism for sulphur. The maximum yield occurred with 20 mg. of sulphur per liter. Analogous values for sulphur supplied as sulphuric acid are almost identical at the lower sulphur concentrations. The first indication of a divergence is at 20 mg. of sulphur per liter. At this point the yields with sulphur as sulphuric acid begin to diminish, presumably as the effect of an excessive increase in acidity.

The pH values at harvest showed a relation similar to those of the yields, whereas the results with sporulation were quite parallel

TABLE I

EFFECT OF ADDITION TO THE NUTRIENT SOLUTION OF SULPHUR UPON GROWTH AND DEVELOPMENT OF A. NIGER

Sulphur mg./L	Sulphuric acid			Sodium sulphide			Sodium sulphate		
	Yield (mg.)	pH	Spores	Yield (mg.)	pH	Spores	Yield (mg.)	pH	Spores
.....	19.3	3.42	1,bl*	18.0	3.14	1,j	19.9	3.12	1,j
5	344.3	2.00	5,bl	.....	.....	.....	353.3	2.00	5,bl
10	616.8	1.80	3,bl	42.2	2.97	2,j	593.1	1.77	5,bl
15	820.2	1.78	3,bl	.....	.....	.....	787.0	1.78	4,bl
20	801.8	1.92	3,bl	63.0	2.75	3,j	881.5	1.82	3,bl
25	781.8	2.03	3,bl	.....	.....	.....	865.6	1.88	3,bl
30	758.8	2.21	3,bl	93.1	2.54	4,j	868.3	1.94	3,bl
35	732.8	2.24	3,bl	.....	.....	.....	864.1	1.88	3,bl
40	703.8	2.31	3,bl	127.8	2.43	5,j	870.4	1.94	3,bl
45	.....	.....	.....	.....	.....	.....	884.1	1.92	3,bl

* Sporulation is indicated as numbers 0 (sterile) to 5 (entirely covered with spores); their color by the initial letter of the shades white, yellow, tan, brown, black, and jet.

for the two series. The ratios of maximum to minimum yield were 44.3 for sodium sulphate and 42.5 for sulphuric acid. These values correspond to a decrease in yield upon non-addition of sulphur of 97.7 and 97.9 per cent from the maximum with sulphur, respectively.

Despite the increases in yield with sodium sulphide, there is no evidence that sulphur in the form of sulphide may participate in the sulphur nutrition of the fungus, although the sulphide ion evidently is innocuous in the concentrations employed. The readiness with which sulphides undergo oxidation renders it probable that traces of sulphate were present in the salt, or were formed during

the course of the experiment. This interpretation is in agreement apparently with the data given in table II.

These statements should not be assumed to imply that only sulphur as sulphate ion is available to the organism. PFEFFER points out in his textbook that not only sulphates but also "isothionic" acid, taurine, and certain sulpho-acids may serve as sources of sulphur even with the higher plants. The data in table II indicate

TABLE II

EFFECT OF BARIUM SALT UPON UTILIZATION OF SULPHUR IN VARIOUS FORMS BY A. NIGER

$BaCl_2 \cdot 2H_2O$ GM./L	40 MG. S/L (H_2SO_4)			30 MG. S/L (Na_2S)			1 MG. S/L (IMPURITY)			40 MG. S/L (CYSTINE)		
	YIELD (MG.)	pH AT HARVEST	SPORES	YIELD (MG.)	pH AT HARVEST	SPORES	YIELD (MG.)	pH AT HARVEST	SPORES	YIELD (MG.)	pH AT HARVEST	SPORES
.....	703.8	2.31	4,bl	93.1	2.54	5,j	19.3	3.42	1,j	889.7	2.15	4,bl
0.1	751.8	2.05	4,bl	80.0	2.73	4,j	20.1	3.18	1,j	829.3	1.92	4,bl
0.2	727.9	1.84	4,bl	59.7	2.93	4,j	19.5	3.18	1,j	814.9	.....	4,bl
0.3	630.1	1.84	4,bl	51.5	2.95	4,j	18.5	3.18	1,j	842.6	1.88	4,bl
0.4	353.4	1.88	5,bl	57.6	3.03	4,j	.....	.....	.....	.....	.....	.....
0.5	317.0	1.98	5,bl	.....	.....	.....	.....	.....	.....	.....	.....	.....

the suitability of cystine as a sulphur supply. Other data on hand have shown sodium thiosulphate to be an excellent source of sulphur for the fungus, while even potassium thiocyanate may be utilized, although with difficulty.

Non-addition of sulphur, moreover, results in the formation of submerged and slimy translucent hyphae that seem unable to form a felt upon the surface of the nutrient solution. Spore formation first increases and then decreases almost to the point of complete suppression with progressive decreases in sulphur concentration.

The effects of the addition of barium salt to the nutrient solution containing sulphur in various forms are shown in table II. Addition of barium salt to the solution containing sulphur as sulphate ion resulted in the formation of a white precipitate which apparently was $BaSO_4$. Small quantities of barium salt brought about slight increases in yield and acidity at harvest, since sulphuric acid was present at more than double the optimum concentration. Larger quantities, however, brought about a progressive decrease in yield and acidity at harvest accompanied by increased sporulation. The

macroscopic appearance of the felts with increasing concentrations of barium salt became more and more similar to those of the minus sulphur, minus iron, and minus zinc cultures. At barium concentrations sufficient for precipitation of all sulphate ion, the barium cultures, the minus sulphur, the minus iron, and the minus zinc cultures are practically indistinguishable in appearance, yield, and final pH.

That chemical precipitation of sulphur in a form relatively unavailable to the fungus is the underlying cause of the action of barium is also borne out by the evident harmlessness of barium in the low sulphur and organic sulphur cultures. The high yields with organic sulphur present in a form not precipitated by barium would indicate moreover that sulphur, once it has penetrated the cells, can be utilized by the fungus despite the presence of barium in the nutrient solution. The progressive decreases in yield and increases in pH at harvest with an increasing concentration of barium salt when sulphur is supplied as sulphide would indicate that growth is due to traces of sulphate.

The absence of any uniformity in response to the various barium salts in McHARGUE's experiments (3) would seem to eliminate nutrition, partial substitution, and ion toxicity as the factors responsible for the increases reported with green plants. That is to say, the increased growth is in all probability due to an improvement in the physicochemical properties of the nutrient solution, and only secondarily to any action on the plants. Sufficient evidence is not available, however, to determine the specific cause, such as increased availability of magnesium, favorable modification in concentration of one or more of the nutrient ions, or complex salt formation, etc. There is every reason to believe that many cations and anions of the unessential elements may function to aid the organism through adjustment of the physicochemical properties of the environment without entering directly into the metabolism of the organism. Such for example is the case cited in this paper in which an increase in growth is accomplished through addition of barium chloride to the nutrient solution.

In nutritional studies with the plant it would seem desirable to employ where possible the optimum solution; that is to say, to uti-

lize a solution containing the necessary nutrients in amount just sufficient for maximum normal development and therefore free from nutrient deficiency or ion toxicity. Partial substitution because of nutrient deficiency also cannot be a factor in the results with the optimum solution. Partial substitution, or rather substitution, since it is *a priori* impossible wholly to replace an essential element in its functions in the protoplasm, has reference here to the actual substitution for the element incorporated into the cell structure of an unessential element or another essential element. Although the distinction has been overlooked, substitutions in the nutrient solutions having the effect of conserving or expediting the utilization of nutrients are also possible and may be referred to as "replacement." One of the ultimate effects of the interplay of substitution, replacement, ion toxicity, and physicochemical modification of the nutrient solution is to increase the optimum ranges of the individual essential inorganic constituents so that maximum growth is capable of being attained despite a marked variation in nutrient proportions and concentration (1).

With these facts in mind it must be admitted that there is more than one nutrient solution with respect to composition that may be properly referred to as optimum in that it permits of the apparently normal maximum development of the organism. The true optimum solution has reference, however, only to one capable of affording a maximum normal development with a minimum of constituents under a definitely prescribed physical environment. Its nutrient proportions are not necessarily absolute since here also substitution and replacement are feasible and may still occur to a limited extent. The true optimum solution is the minimum balanced diet required for maximum normal development. Its probable variation in absolute concentration with variation in temperature and other physical factors, moreover, does not detract from its usefulness, if experience in animal nutrition is a satisfactory criterion.

Use of an optimum solution containing a minimum of constituents possesses several advantages experimentally over that of other optimum solutions. An optimum solution composed of nutrients in uselessly excessive quantities leads to the introduction of increased quantities of the ever present impurities they contain. In the pres-

ence of an excess of nutrient compounds maximum growth occurs only because any ion toxicities due to the individual components mutually cancel or mask each other. Also there is as yet no proof that a solution physiologically balanced with respect to a single function is necessarily balanced with respect to all functions, so that the use of needlessly excessive concentrations should be avoided. Because of the decrease in ion toxicities of the individual constituents with decreasing concentration, these effects will be at a minimum, in general, in the optimum solution which is most dilute. It follows moreover that ion toxicities that may arise from depletion of the nutrient solution will also be at a minimum.

It is obvious that in the experiments herein reported, the formation of translucent and watery hyphae in the absence of sulphur is a morphological abnormality due to nutrient deficiency. Other data on hand afford proof that a deficiency of nitrogen, phosphorus, magnesium, iron, or zinc also leads to similar results. It may be concluded therefore that the morphological characteristics of the control or so-called "unstimulated," "normal," or minus zinc culture usually obtained in zinc stimulation studies are really the abnormal, whereas those of the "stimulated," or zinc culture, are actually the normal. The barium effect within the range studied is due to the removal of sulphur.

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DAILY VARIATION IN THE SENSITIVITY OF MIMOSA WITH SPECIAL REFERENCE TO THE ACTION OF LIGHT

W. E. BURGE, G. C. WICKWIRE, AND H. J. FULLER

(WITH TWO FIGURES)

As is well known, the leguminous plant *Mimosa* responds to a variety of external stimuli with an infolding of the secondary leaflets and a fall of the main petiole. The present investigation was begun to determine what effect, if any, daylight and darkness would have on this capacity; in other words, to determine the effect of light and darkness on the sensitivity or irritability of *Mimosa* when the temperature effect was excluded by keeping this constant.

Vigorous greenhouse plants about 18 cm. tall were used. These plants were grown from seed sown in 4-inch pots in April, 1934, and were kept throughout the experiments in a well-lighted east greenhouse. Some of the experiments were performed in September and October before the onset of cold weather, others in November and December. During the latter months the greenhouse was maintained at a temperature of approximately 26°–27° C. The degree of irritability was ascertained by determining the weakest stimulus that would cause a downward movement of the main petiole. Later it is proposed to use chronaxie as a measure of irritability, instead of minimal or threshold stimulus as was done in this investigation, when a comparison of the irritability of *Mimosa* will be made with that of other plants and animal tissues. The method of stimulation was to drop cylindrical pieces of wood, 3 mm. in diameter and of various lengths, through a glass tube 25 cm. long with a bore of 7 mm., so that they would strike the plant at the junction of the four primary leaflets as shown in figure 1.

One leaf of each of nine plants was selected for stimulation. At 6 o'clock in the morning, which is about daybreak at the first of December, the stimulations were carried out, beginning with the smallest weight (15 mg.) and using the other weights in rapid suc-

cession in the ascending order. In this way the weakest stimulus was determined which would cause a drop of the leaves. Three hours later, at 9 A.M., the same procedure was repeated, and again at noon, 3 P.M., 6 P.M., 9 P.M., midnight, 3 A.M., and 6 A.M. the next day.

FIG. 1.—Method of procedure in dropping weights through tube to stimulate the plant, *Mimosa*.

The continuous line curve shown in figure 2 was constructed from the average of the data obtained from the nine plants. The weights in milligrams of the cylindrical pieces of wood that were found to be minimal or threshold stimuli are the ordinates while the times of day these weights were effective in producing a drop of the leaves are the abscissas. It will be seen that at 6 A.M. a weight of 68 mg. was required to produce a drop of the leaves while at 9 A.M. a weight of 35 mg. was sufficient. This response is taken to mean that irritability of the leaves had risen between 6 and 9 o'clock in the morning. The fact that relatively weak stimuli evoked a response of the leaves throughout the remainder of the day is likewise interpreted to mean that irritability remained relatively high during the day. It may be seen further (fig. 2) that as the evening came on, stronger stimuli were required to evoke a response, until the following morning at

6 A.M. when a 65 mg. weight was required. Apparently irritability gradually decreased during the night to the original low ebb around 6 A.M., approximately the same as it was on the preceding morning when the experiment was begun.

It should be mentioned in this connection that a considerable difference was found in the degree of irritability of the nine plants used, but all of them showed the same abrupt rise in irritability during the first part of the morning, which remained high during the day and fell gradually at night.

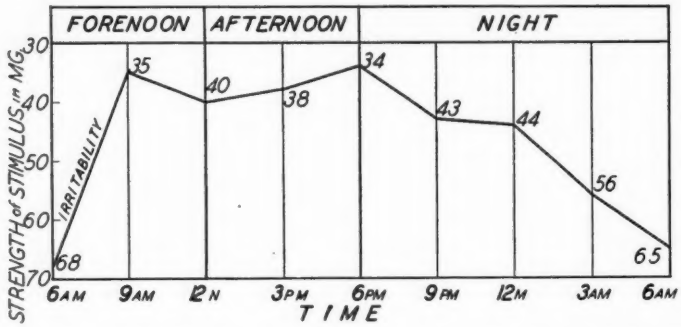

FIG. 2.—Curve illustrating that the irritability of *Mimosa* rises rapidly during the first part of the morning, remains high during the day, and falls gradually during the night.

This increase in irritability suggested that light might be responsible, and in order to determine whether this was true, two series of experiments were carried out. In one series the plants were illuminated continuously for 24 hours with a 300 watt Mazda lamp at a distance of 60 cm., and in the other series the plants were kept in darkness for a similar period of time. It was found that the irritability of the plants in the light remained at the high daytime level while the irritability of these same plants when kept in darkness remained at the low nighttime level. When the plants that had been kept in the dark were transferred to the light the irritability rose and remained at the high daytime level, and when the plants that had been kept in the light were transferred to darkness, the irritability dropped and remained at the low nighttime level.

These observations are directly contradictory to the results reported in the literature. WALLACE (7) observed that the irritability of *Mimosa* was highest around 5 o'clock in the morning, a time when we found it was lowest; that it diminished during the day when we found it was increased, and increased during the night when we found it was diminished. A possible explanation for this contradiction may be found in the different criterion used for measuring irritability. WALLACE, like other investigators in this field, used extent of movement (angle of fall) of the leaf as a measure of irritability while we used the minimal or threshold stimulus. Hence it would seem to be in order at this juncture to look into the merits of the criteria used.

The drop of the leaf of *Mimosa*, when stimulated, is due to the loss of water from the parenchymatous cells on the lower side of the pulvinus. What the stimulus does is to cause these cells to give up water, as a result of which the petiole falls. Hence it seems that the degree of drop of the leaf of *Mimosa* is an index to water loss rather than to irritability. Irritability in this case is the *capacity* of the leaf to respond to the stimulus, while water interchange is connected with another property, namely turgidity, which is involved in the actual *response*. In the works of WALLACE and others, the degree of response, not the capacity to respond, has been measured and has been (erroneously, it seems to the writers) taken as the criterion of the capacity to respond.

WALLACE (6) also used the extent of movement of the leaf of *Mimosa* as a measure of irritability in his study on the influence of chloroform, ether, nitrous oxide, ethylene, various other chemicals, temperature, age, etc. BOSE (1) and the earlier workers on *Mimosa* used the extent of movement of the petiole as a measure of irritability, although in most cases quantitative measurements are rare. CORRENS (3), in his work on the relation of free oxygen to responses in *Mimosa*, used the fall of the main petiole and the degree of folding of secondary leaflets as the indices of sensitivity. PRINGSHEIM (5) writes, "External factors which lower the sensitivity [of *Mimosa*] without killing the plant, can affect the plant in such a way that a given contact stimulus may initiate a response of only half the normal extent." MACDOUGAL (4) states, "The angles [of petioles with

the main stem] should be measured exactly with a protractor," in a discussion on irritability and response in *Mimosa*.

It is recognized that the extent of muscular contraction is not a measure of irritability but of contractility, and that the lowering of temperature of a muscle decreases its contractility but increases its irritability. Likewise, the suspending of a weight from a leaf of *Mimosa* decreases the extent of movement of the leaf but does not decrease its irritability (2).

A priori, it is difficult to understand why the irritability of *Mimosa*, or the susceptibility of this plant to the action of stimuli, should be at its lowest ebb during the day, a time when the activity of the plant is highest and it is being most intensely stimulated, and irritability highest at night, a time when the plant is being least intensely stimulated. It would seem that the reverse should be true, as is found to be the case when threshold stimulus is used as a measure of irritability rather than extent of movement.

Summary

1. The irritability of *Mimosa pudica* rises rapidly during the first part of the morning, remains high throughout the daylight hours, and falls gradually during the night to a minimum around daybreak.

2. If plants are kept under continuous illumination for 24 hours, irritability does not fall during the night period as normally occurs but remains at the high daytime level.

3. If plants are kept in the dark for 24 hours, irritability does not rise during the daytime period as normally occurs but remains at the low nighttime level.

4. It is concluded that the increase in irritability during the day is due primarily to the effect of light.

5. Other investigators have found contrary to our observations that the irritability of *Mimosa* is lowest in the daytime and highest in the night. This contradiction in results is attributed to the use by other observers of an improper criterion and method for determining irritability.

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DOUBLE INTERLOCKING OF BIVALENT CHROMOSOMES IN LILIUM ELEGANS[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 469

J. M. BEAL

(WITH TWO FIGURES)

Interlocking of bivalent chromosomes during meiosis appears to be of somewhat frequent occurrence in both plants and animals. SAX and ANDERSON (5) reviewed the cases known up to 1934, and discussed them with relation to their bearing upon the classical and partial chiasmatypy theories. All the cases then known were of the single type and the great majority of these showed proximal interlocking. The high frequency of proximal interlocking, together with the infrequency of distal interlocking, was used as an argument in support of the classical theory of chiasmatypy. Furthermore, at this time interlocking was known only in genera in which segmental interchange is commonly found. Other cases have since been found in genera in which segmental interchange is unknown. In an analysis of ten metaphase bivalents in *Lilium* showing single interlocking, MATHER (4) found three examples of interlocking in the attachment or proximal loop, five in the loop next the attachment loop, and two in the loop next but one to the attachment loop. As MATHER points out, since interlocking is dependent upon pairing between the homologous chromosomes, the type of pairing is very important. Pairing may begin (1) at random along the length of the chromosomes when the nucleus is not polarized, or (2) at the ends when the nucleus shows polarization. In *Tradescantia*, in which interlocking is common (5), the nucleus is polarized. Pairing thus begins at the ends of the chromosomes and chiasma formation occurs early there. This appears to inhibit chiasma formation in the median region of the paired chromosomes. ANDERSON and SAX (1) now state that this interpretation of chromosome pairing explains the prevalence of

[1] This investigation was aided in part by a grant to the University of Chicago from the Rockefeller Foundation.

proximal interlocking in *Tradescantia* and invalidates the data previously cited (5) as evidence in favor of the classical theory.

There is no evidence of polarization in *Lilium*, hence pairing and interlocking (when it occurs) should be at random. And since there is little terminalization of chiasmata in *Lilium*, the position of the interlocked chromosomes should show little change during the later prophase stages. This assumption is supported by the observations of MATHER on the cases of single interlocking in *Lilium*, as well as by the known cases of double interlocking. Double interlocking, on the other hand, appears to be extremely rare, since only two examples

Figs. 1, 2.—Fig. 1 (left), double interlocking between the two bivalents of *Lilium elegans*. Both chromosomes of bivalent A are interlocked in two adjacent internodes of bivalent B. ×ca.2400. Fig. 2 (right), probable chromatid relations in the two interlocked bivalents of figure 1, based on the partial chiasmatypy theory.

have been reported previously. The first of these was described by MATHER (3) for *Lilium regale* and the second by UPCOTT (6) for *Eremurus*. A third case can now be recorded.

During the examination of smear preparations of the pollen mother cells of *Lilium elegans*, fixed in Flemming's medium solution and stained in accordance with Newton's gentian violet-iodine method, a single example of double interlocking was observed (fig. 1). Both homologous chromosomes of bivalent A are interlocked between the two chromosomes of bivalent B, one chromosome of A being interlocked in the attachment or proximal loop of B and the other in the loop next the attachment loop. A chiasma is present in bivalent B between the two loops, and each of the interlocked bivalents shows a total of three chiasmata.

During the zygotene-pachytene pairing both chromosomes of bivalent A must have been caught at some distance apart between the two pairing chromosomes of bivalent B. The formation of a chiasma by the two chromosomes of bivalent B between the two chromosomes of bivalent A effectively prevented the latter two

chromosomes coming together at this point. Figure 2 shows an analysis of the probable chromatid relations of figure 1.

MATHER (4), in a study of several species of *Lilium*, has shown that there is little reduction in the number of chiasmata from diplotene to metaphase. *Lilium elegans* shows little reduction, and it is highly improbable that two nodes or two chiasmata had been present during diplotene in bivalent B between the two interlocked chromosomes of bivalent A. DARLINGTON (2) has pointed out that paired chromosomes remain paired at diplotene by both chiasmata and relational coiling. It is often difficult to distinguish between these two conditions during early diplotene and no doubt relational coiling has often been confused with chiasmata, especially in those cases where chiasmata are formed at random. In *Fritillaria* species in which chiasmata are localized, and are thus more easily recognized, there is practically no reduction in the number of chiasmata between diplotene and metaphase (2). In *Lilium*, with random chiasmata, the apparent reduction in the number of chiasmata may be explained on the basis of the disappearance of relational coiling rather than as a result of terminalization or breaking of chiasmata, since there is slight terminalization and little or probably no breaking of chiasmata.

It is therefore highly probable that a single chiasma was formed in bivalent B between the interlocked chromosomes of bivalent A and that no reduction or breaking of chiasmata has occurred. If this assumption is true, then the arrangement of the chromatids as shown in figure 2 must be true and adds strong support to the partial chiasmatypy theory of crossing-over.

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CURRENT LITERATURE

Pollen Grains: Their Structure, Identification, and Significance in Science and Medicine. By R. P. WODEHOUSE. New York: McGraw-Hill, 1935. Pp. xvi+574. Illustrated. $6.00.

The first comprehensive study of pollen grains to appear in the English language is welcomed by pollen analysts and hay fever specialists. Morphologists and taxonomists may also consult the work with profit since, in addition to that drawn from the author's earlier papers, much new material is included to prove the thesis that "in no other part of the plant are to be found packed in so small a space so many readily available phylogenetic characters." The approach is therefore on the basis of comparative morphology.

The volume falls into two sections. Part I includes the following chapters: historical review; methods of collecting pollen in large amounts; preparation of pollen for microscopic examination; pollen statistics: a botanical and geological research method by G. ERDTMAN; atmospheric pollen (at Yonkers, New York); hay fever; and pollen grain characters. Eighty-five pages are taken up by the historical review, and the contributions of VON MOHL, FRITZSCHE, and HUGO FISCHER are described in detail.

The chapter on pollen grain characters forms the necessary morphological background for part II. The author points out that "while heredity or phylogeny tends to dominate the basic form of pollen grains, the internal environment tends to control the number and arrangement of their germinal furrows and pores, and the external environment (wind *versus* insect pollination) tends to modify their sculpturing." His treatment of the last point seems somewhat teleological throughout the book. The various furrow patterns are subjected to mathematical analysis and most of them are seen to fall into the trischistoclasic system.

The section on classification is headed with a key to 41 angiosperm families or their representatives, and to the seven orders of gymnosperms. The text material which fills the remainder of the volume presents specific, generic, and family descriptions, and keys to certain genera and species. Thirty-five genera of living gymnosperms, representative fossil forms, and 31 angiosperm families are treated. These, according to the author, "fit together, quite naturally, in a sequence corresponding, for the most part, to the system of ENGLER and PRANTL." The evidence from pollen morphology for this sequence of families in the dicotyledons seems inconclusive, since the basic plan is common throughout, and many of its variations are repeated in families which are widely separated in the Englerian system. The major departure from this system involves

the position of the Magnoliaceae as the primitive family. According to WODEHOUSE, the members of the Magnolieae show single-furrowed pollen grains which are virtually indistinguishable from those of the Bennettitales. He derives the tricolpate grain of nearly all other dicotyledons from the primitive monocolpate grain through the anomalous pollen of *Schizandra* and *Kadsura*. These two genera exhibit "side by side, the principal features of the triradiate pteridophyte spore, the monocolpate gymnosperm pollen grain, and the trischistoclasic dicotyledonous pollen grain."

Other phylogenetic interpretations based on pollen characters include the following ideas. The Nymphaeaceae, the monocotyledons, and the six tribes of the Coniferales, in addition to the Magnoliaceae, may be regarded as coordinate genetic lines. These types inherited the broad open type of furrow characteristic of the Cycadales, *Ginkgo*, and the Bennettitales. Further pollen grain evolution along these genetic lines has centered in means of modifying, protecting, or eliminating this furrow. With regard to the evolution within the Coniferales, WODEHOUSE's interpretations are suggestive. He believes that the Taxodineae and the Cupressineae originated from the cordaitalean stock independently of the Abietineae, and that the Taxineae are closely related to them. The Podocarpineae are most closely related to the Araucarineae, although *Araucaria* and *Agathis* probably represent the end of a distinct line of development. His treatment of the monocotyledons is very brief, although the pollen of the Palmaceae is thought to be the most primitive of the forms discussed. In spite of this, he places this family after the Typhaceae and Naiadaceae in his sequence of families.

As one might expect in a work of this size, the book is not free from error. Thus one finds that *Welwitschia* exists in southeast Africa, and that beech pollen has not been recorded from bog deposits in America. *Digitaria* is placed in the generic sequence of the Festuceae, and synonyms are sometimes used indiscriminately. One may also consult the bibliography in vain for certain citations in the text.

But these points are minor. The beauty and clearness of the numerous plate and text figures, the lucid and concise descriptions, and the suggestive phylogenetic interpretations make this book a volume which many botanists and physicians will wish for their libraries.—C. E. OLMSTED.

Vergleichende Morphologie der höheren Pflanzen. By WILHELM TROLL. Vol. I. Part I. Vegetationsorgane. Berlin: Gebrüder Borntraeger, 1935. Pp. 172. With 104 illustrations.

The initial section of the first volume of a comprehensive German work on the comparative morphology of the higher plants has recently been published. The author, Dr. WILHELM TROLL, professor of botany at Martin Luther University, Halle, Wittenberg, has outlined the scope of the complete work in the foreword. It will consist of three volumes of several numbers each, which will

be published at regular intervals. The first volume, in addition to the introductory chapters, will be devoted to the morphology of the vegetative organs while the remaining two will deal with the comparative morphology of the inflorescence, including flower, fruit, and seed development. Some consideration will be given to the vegetative organs of the pteridophytes, but the major emphasis will be upon the seed plants.

Part I of the first volume includes an introductory section, and the first main section of the work which is concerned with the gross morphology of the vegetative organs of the seed plant. In the introductory passages general biological concepts are discussed, including sections on homology, analogy, and convergence, teratology, organography, developmental and experimental morphology, and phylogeny. The main section of 25 chapters deals with the gross morphology of the higher plants, and selected examples of common plants are used as illustrative material. Representative chapters are those on the seed, embryo, and development of the root of the radish; development of the leaf of the pea; seedling and shoot development in corn; the development of the onion; tuber formation in the potato; and others dealing with buds, bud scales, and bud development, phyllotaxy, seed germination, bulb formation, and root and shoot development.

The selection of common native and economic plants as examples and for illustrative purposes is most desirable and will undoubtedly add to the value of the work. It is refreshing to find this tendency to use well known plants appearing in morphological studies; they have too long been devoted to elaborate investigations of remote and little known plants.

An accurate evaluation of this work must await the appearance of succeeding sections as the initial one is introductory and general in character. The real worth of the forthcoming parts will depend in large measure upon the nature of the detailed morphology included and the extent to which dynamic and developmental phases of plant anatomy are stressed.—H. E. HAYWARD.

Protoplasm. By WILLIAM SEIFRIZ. New York: McGraw-Hill, 1936. Pp. x+583. $6.00.

About a century ago biologists began to recognize the slimy material which they observed within the cells of organisms as living substance. The term protoplasm to designate the fundamental living material was first used a little over ninety years ago, but did not become popular until VON MOHL gave it prominence in connection with his investigations in 1846. Many of the great biological speculations have developed from the contemplation of the ultimate nature and intimate structure of protoplasm and from attempts to explain the transmission of hereditary qualities from generation to generation. Such units as biogens, bioblasts, gemmules, pangens, plastidules, biophores, genes, micellae, etc., have been postulated by the speculative biological philosophers of the nineteenth century. But aside from philosophical speculations,

a vast store of information has been accumulated by investigators who have taken protoplasm as a concrete but variable material to be studied by every means known to man. Physico-chemical methods and studies of matter in the colloidal state have thrown a flood of light upon the behavior and nature of this most remarkable substance in the universe.

The important advances which these investigations have made possible are summarized by SEIFRIZ in a book that students of biology will welcome. The principal emphasis of the volume is placed upon the physical and physico-chemical properties of protoplasm. The early chapters consider the general properties of living protoplasm, its organization into cells, and methods of studying its properties and behavior, such as micrurgy and tissue culture. The colloidal properties and structure of protoplasm naturally receive a great deal of attention in chapters on the colloidal state, emulsions, hydrophilic sols and gels, and in the consideration of such properties as surface tension, adsorption, osmosis, imbibition, viscosity, elasticity, and permeability to water, ions, and molecules.

In later chapters acidity, electrophysiology, electrokinetic phenomena, and the influences of radiant energy are summarized. These are followed by a consideration of the role of water, salts, and the food materials, carbohydrates, fats, and proteins. A brief chapter covers the regulatory mechanisms, enzymes, hormones, vitamins, pigments, and other regulatory substances of animal and plant protoplasms. In places the information will seem meager, but the author could not have kept himself within the limits usually imposed if the treatment were adequate at all points. A final chapter deals with the origin of living matter, a problem that will probably remain with biologists for many years as a challenge to more fundamental investigation. In connection with the reference to BENJAMIN MOORE, one misses consideration of the recent papers of FRANCIS which continue the ideas that MOORE first expressed and illustrate them with many contributory observations. The bibliography of choice selections for collateral reading on the various chapters will be helpful to students.

The book is written primarily for students, is not too technical, and is an interesting and valuable contribution which will bring pleasure and stimulation to those who are fortunate enough to have the privilege of reading and digesting the abundance of information presented.—C. A. SHULL.

Ugressfrø (*Unkrautsamen, Weed Seeds*). By EMIL KORSMO. Oslo, Norway: Grøndahl and Sons Boktrykkeri, 1935. Illustrated.

This recent book by EMIL KORSMO is destined to aid materially in weed and seed investigations. It contains a wealth of material, representing the culminating effort of a lifetime devoted to a subject that in most countries has been somewhat neglected.

The book contains descriptions and illustrations of 306 plant species characterized as weeds. The species included are those found commonly in east,

west, central and north Europe, and less commonly in south Europe and North America. There are 34 full-page colored plates, each with illustrations of nine species. Each illustration in clear detail includes all or part of the inflorescence; a fruit depicting the method of dehiscence (for dehiscent fruits); natural and enlarged views of the seed; and finally transverse and longitudinal sections of the seed showing the position and relative size or thickness of the endosperm, embryo, and seed coats. The illustrations are all in natural colors, although it must be recognized that the colors exhibited by many species are not the same in every geographical area. Preceding each plate is a description in Norwegian, German, and English of each species figured. The description covers the type of inflorescence, kind of fruit (silicle, achene, capsule, etc.), shape and size of seed, color and appearance of the seed coat; and the distribution, common habitat, and methods of seed dissemination. The species are arranged according to what the author calls life forms, designated by symbols (annuals, winter annuals, biennials to perennials, and perennials with rootstocks). Within each group the species are arranged in families. From the standpoint of the teacher or the taxonomist, the major grouping is somewhat unsatisfactory, but the aim of the author has been to serve the agriculturist as well as the botanist, recognizing at the same time that the life history of a given species is affected by geographical distribution. The index lists alphabetically the botanical names of all the species together with their common names, in eleven languages. The book will doubtless find a place on the reference shelves of those who teach courses dealing with weeds and seeds, and will also serve as a material aid to the investigator and the seed analyst.—R. H. PORTER and T. E. MELHUS.

Tree Flowers of Forest, Park, and Street. By WALTER E. ROGERS. Appleton, Wisconsin: Walter E. Rogers, Lawrence College, 1935. Pp. 500. Illustrated.

This is the only work of its kind known to the reviewer. By means of many handsome plates, done in a "duotone green," the flowers of our common trees are shown on a large scale of magnification and with a fidelity to nature seldom equaled in the whole domain of floral literature. The book "is intended to be and is chiefly pictorial," therefore the textual material is mostly of a supplementary and simplified nature. By means of many skillfully drawn silhouettes, the author portrays in an ingenious way the deciduous trees in their winter state of defoliation. Art teachers and botany teachers will undoubtedly join in praise of this outstanding feature, since it affords a most effective way of imparting to students instantly a realization of some of the trees' major characteristics. Students of conventional design will find an abundance of source material on which to draw for application to the needs encountered in the practical arts. Much of this material has hitherto escaped the notice of all but botanists, for the very reason that it was of diminutive size.

The mechanical features are of the highest order and combine to make a truly sumptuous volume, the publication of which represents a landmark of achievement for the science of botany in America.—E. E. SHERFF.

Die Pilze Mitteleuropas. Editorship of H. KNIEP, P. CLAUSSEN, and J. BASZ. Leipzig: Werner Klinghardt, 1935.

Die Pilze Mitteleuropas has been appearing in serial form. The fifteenth issue of volume I has appeared, containing the Boletaceae by KALLENBACH. In the meantime volume II has been begun with three issues. These are devoted to Tremellineae by NEUHOFF, and Lactari by KNAUTH and NEUHOFF. The new issues continue on the high level previously established. The publisher announces a reduction of 25 per cent in the price to foreign subscribers.—G. K. K. LINK.

Die Pflanzenzelle—Vorlesungen über normale und pathologische Zytomorphologie und Zytogenese. By E. KÜSTER. Jena: Fischer, 1935. Pp. xii+672. Illustrated.

This volume by KÜSTER is a notable contribution not only to botany but to other biologic sciences as well. It is a synthesis of a vast literature by one who has enriched it significantly through several decades. Its value is enhanced by critical evaluations of present trends and by suggestions of promising investigations. One is also permitted stimulating glimpses of the author's view on theoretical biology. The volume deals with both normal and abnormal plant cell morphology and cytogenesis. These correlative aspects of cellular life are so well integrated that their essential unity is apparent at all times. For this reason the volume will prove interesting and useful both to cytologists and to pathologists in the botanical and zoological fields.

Inclusion of the normal and anomalous in one volume should do much to break down the artificial barriers that have tended, since the last quarter of the 19th century, to segregate as independent biological provinces the normal and healthy on the one hand and the anomalous and pathic on the other. This separation, warranted at one stage in biology because it formulated and focused attention on specific problems, has outlived its usefulness. There has always been difficulty and confusion in delimitation of the pathologic. Some of the difficulties are presented in the introductory discussions of KÜSTER's well known earlier volumes, Pathologische Pflanzenanatomie and Pathologie der Pflanzenzelle. In general the pathologic has been equated with (a) the statistically abnormal, (b) the abnormally induced, (c) harmful abnormality, (d) destruction and the dysfunctional, that is, harmful events (injuries) at or between the surfaces of organisms and their parts, irrespective of the normality or abnormality of their occurrence or causation.

KÜSTER defines the field of the pathologic as comprising all events due to anomalous conditions and agents, both internal and external. The pathologic accordingly includes all experimentally induced effects.

To consider as pathologic any event due to statistically abnormal causes is essentially the same as defining it as a statistically anomalous event. The discovery that variation is a pervasive characteristic of living things seems to indicate that this criterion of the pathologic is too inclusive.

Considered from another angle the criterion is too exclusive. Unreserved exclusion of the statistically normal from the field of the pathologic bars many events that constitute either destruction (necrosis is a special case) or functional impairment (failure to adapt and to survive is an extreme case) at one or more levels of biologic organization. In the reviewer's opinion, if only one criterion for the pathologic is sought, it should be the criterion of harm or injury in the broadest sense of the term. Thus any event is pathologic that actually or potentially destroys or functionally impairs any given system as a whole, or any part of such system. Strictly speaking, a harmful event (injury) plus the train of reactions incited by it constitutes the complex known as disease. Injury and reaction to injury accordingly constitute the center of the concept pathologic, while the anomalous is its fringe. This definition includes both normal and abnormal injuries and injury reactions in the field of the pathologic.

KÜSTER unreservedly accepts as pathologic all events which stand in a causal relation to death. Nevertheless he chooses to designate them physiologic if they are statistically normal. He speaks of physiologic degeneration, destruction, death, etc. Granted that natural, normal, regular, and similar statistical concepts are some of the ancient meanings of the physiologic, is it not advisable for the sake of greater precision to abandon this usage together with that other misnomer, physiologic disease, which is so frequently and confusedly used by botanists? Formulation of the physiologic-pathologic antithesis in causal terms has done much to engross plant and animal pathologists in consideration of causal agents, especially parasites, with attendant neglect of the material or functional disturbances of the affected system. In most American botanical textbooks the concepts abnormal, pathologic, and disease are equated and introduced with and limited to discussions of parasitic bacteria and fungi. If physiologic and pathologic are to be used as antithetic terms, is it not preferable to give them a teleologic content, for which there is ample precedent, and designate as physiologic those events which are functional with reference to the system under consideration, including keeping itself adapted and alive, and as pathologic the destructive and dysfunctional ones?

The subject matter of the volume is treated in seven chapters: Protoplasm, nucleus, plastids, vacuoles, starch, crystals and dead inclusion bodies, wall, and cytogenesis. By choice caryology is less completely treated than the other topics. In each chapter discussion of the abnormal and pathologic immediately follows presentation of the normal and healthy.

Protoplasm (cytoplasm in the sense of STRASBURGER), nucleus, and plastids are recognized as three forms of living matter of cells, while vacuoles, inclusion bodies such as starch, and the wall are considered dead products of the living substance. There is no hint as to whether genes are considered living or nonliving, or as antecedents or products of other cellular substances. The dead parts of cells are recognized as constituents of the living continuum known as the protoplast. KÜSTER accepts the proposition that protoplasm is fluid and characterized by sub-microscopic structure. He points out that in the last analysis

all protoplastic activities, including self-regulation, are in some way based on and involve this sub-microscopic structure. Its irreversible destruction is the essence of partial or total protoplastic necrosis. Lesser material and functional injuries, including loss of self-regulation, undoubtedly involve alteration of this structure. In discussing the problem of plasmodesmata, KÜSTER takes a very guarded stand and points out that these structures are not essential for correlation between protoplasts. The proposition that chondriosomes are stages in development of plastids is rejected. KÜSTER does not consider convincing the evidence advanced for considering them living substances.

The chapter on cytogenesis includes an interesting discussion of the characteristics of living substances. KÜSTER emphasizes assimilative activity and division, but points out that possibly respiratory activity may prove to be a basic criterion of living substance. This chapter also includes a discussion of problems of individuality of cells and of multicellular wholes, of correlation, of senescence, and of death.

KÜSTER subscribes to the proposition that the living substance of unicellulars is potentially immortal provided it is given opportunity to grow and to divide, and is not poisoned by its environment. He does not define as death the destruction of individuality of a protoplast incident to its division. Since most protoplasts of multicellular organisms soon lose the opportunity for division, they become so senescent that death is unavoidable. Senescence is assumed to proceed more or less rapidly in every protoplast and is attributed to hysteretic changes in the protoplastic colloids.

These and other discussions in the chapter imply that the concepts health and disease not only are relative but correlative as well. Instead of asking, "Is this living protoplast healthy or diseased?" one should ask, "To what degree is it healthy and pathic?" KÜSTER's statement, "The physiology of senescence and the pathology of the cell are not separable" is a modern version of ARISTOTLE's statement, "Indeed we may rightly call disease an acquired old age, old age a natural disease," and of CLAUDE BERNARD's aphorism, "The healthy are really invalids unaware of their illness."

While reading this excellent volume, the reviewer repeatedly wished that the time might soon come when biologists would make some agreement as to usage of such basic concepts as cell, protoplasm, wall, membrane, and the like.

Each chapter closes with an excellent bibliography. An authors' and a general index close the volume. The style is easy and clear and the material is well organized. The volume, copiously and excellently illustrated, is printed on good paper and well composed. It is a credit to the author and publisher, and a rich contribution to biology.—G. K. K. LINK.